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ACUTE AND CHRONIC TOXICITY OF ZINC TO THE GUPPY, *POECILIA RETICULATA*

by

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ABSTRACT

Ten-day LC50 values were determined for adult and immature guppies under static test conditions at 25°C (pH, 7.4; total hardness, 95 mg/l $CaCO_3$) to compare their tolerances to zinc. Adult pairs were then chronically exposed to sublethal concentrations of zinc for a 4-month period and the effects on reproduction studied. During this period, motor activity of the females and the intensity of courtship behaviour of the males were measured; the former by the number of times the lines of a grid were crossed during a 5-minute observation period and the latter by the number of times the following displays occurred during a 3-minute observation period: gonopodial swinging, dorsal fin erection, sigmoid reaction. The effect of prenatal exposure to zinc on the tolerance of young guppies to an acutely toxic zinc concentration (6mg/l) was examined by LT50 determinations.

The 10-day LC50 values for adults and immatures were 4.83 and 2.65 mg/l zinc respectively. This difference in tolerance was significant.

Over the four parturition periods, chronic exposure to 0.88 and 1.70 mg/l zinc caused significant reductions in the total number of young produced per female (58% and 50% respectively) and in the length of young (12% and 13% respectively) at birth. Significant decreases in brood sizes did not occur until the second parturition whereas decreases in the length of young at birth occurred as early as the first parturition. Chronic exposure to zinc did not

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significantly affect the time intervals between broods.

Chronic exposure to zinc significantly reduced motor activity, the total activity of females exposed to 0.36, 0.88 and 1.70 mg/l zinc being only 65%, 53% and 38% respectively of the value for control females. Decreased activity of exposed fish occurred as early as the first 3-week interval. The intensity of courtship by males was not affected by zinc.

Prenatal exposure to zinc did not significantly increase LT50 values.

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INTRODUCTION

Low levels of zinc (between 10-200 μg/l) are common in the fresh water environment (Kehoe *et al.*, 1944; O'Connor *et al.*, 1964). These are thought to be the result of weathering and erosion of ore deposits (Huff, 1944; Whitney and Risby, 1975; Williams *et al.*, 1976). The presence of zinc concentrations that are much higher than these levels (Jones, 1940; Newton, 1944; Sprague *et al.*, 1965; Kopp and Kroner, 1967; Saunders and Sprague, 1967) poses serious pollution problems that could damage aquatic ecosystems. Weathering of mine refuse heaps; effluents from ore processing and other zinc industries such as those involved in zinc coating, rubber and paint manufacturing, and paper processing are the major sources of zinc pollution.

The toxicity of zinc to fish and other aquatic life has been studied since the beginning of this century. These studies indicate the probable mechanisms of the toxic action of the metal and also guide authorities in the development of water quality criteria to conserve aquatic life.

In fish, acute zinc toxicity (Sprague, 1969) is thought to be due to tissue hypoxia. Two views are held on events that lead to the development of hypoxic conditions. The first is that the metal causes excessive mucus accumulation on the gill surface (Carpenter, 1927; Ellis, 1937) and thus decreases the permeability of the gill epithelium to oxygen (Westfall, 1945). It has been suggested (Cusick, 1967; Hodson, 1974) that the secretion of mucus might be a response

that protects the gill tissue from damage. Lloyd (1960) found no evidence of mucus accumulation on the gills of rainbow trout, *Salmo gairdneri*, killed by zinc. The second view (Lloyd, 1960; Skidmore and Tovell, 1972; Matthiessen and Brafield, 1973) is that the metal decreases the efficiency of gas exchange by interfering with the structural integrity of the secondary lamellae. Our understanding of the mode of toxic action of zinc is still incomplete. Most of the studies involved unrealistically high zinc concentrations. The possibility exists that the mode of action varies with the concentration of the toxicant.

Much of the literature is on acute toxicity. In these studies, the tolerance of a species is measured by determining an LC50 (the concentration which kills 50 per cent of the animals) for a specified period of exposure and test conditions. The response of the average fish is preferred to that of either the least or the most tolerant individuals since a much smaller variability exists (Sprague, 1969) and values that are obtained are more likely to be representative of the population and therefore more reproducible. The values, that have been reported as acutely toxic, range from 0.09 mg/l for cutthroat trout, *Salmo clarki*, (Sappington, 1969) to 40.9 mg/l for bluegills, *Lepomis macrochirus*, (Pickering and Henderson, 1966). Part of the variation may be accounted for by tolerance differences among species, differences in length of exposure periods, size and number of test animals, and use of different salts of the metal. Differences in the physical and chemical

qualities of the test water account for much of the variation observed. Generally, the water properties that affect zinc toxicity appear to do so by altering either the rate and/or volume of ventilation (Chapman, 1973) and therefore increase the amount of toxicant that reaches the respiratory epithelium; or the biological availability (for example, by decreasing the amount of zinc in solution or by reducing the ease of entry into the gill tissue) of the toxicant.

Hardness is the most important property of water that affects zinc toxicity (Lloyd, 1960), the toxicity decreasing with increase in hardness (Jones, 1938a; Cairns and Scheier, 1957; Lloyd, 1960; Mount, 1966; Sinley *et al.*, 1974; Solbe, 1974). The mechanism of the antagonistic action of calcium and the other alkaline-earth metals (magnesium, strontium, barium) is still poorly understood. Taking into consideration the permeability theory, attributed by Jones (1938b) to the work of Loeb, and Osterhout, researchers speculate that the antagonistic effect might be due to a reduction in the permeability of surface membranes to zinc; probably as a consequence of competition for binding sites on the membranes (Cusick, 1967). The inhibition of zinc-induced precipitation of protoplasm within the cells of fish might be another mechanism (Heilbrunn, 1937).

Another water property that affects zinc toxicity is temperature, probably because of its effects on metabolism and the diffusion rate of materials. Lloyd (1960) observed that although the survival time of rainbow trout in zinc decreased

with increase in temperature, the threshold concentration (the concentration below which zinc is not toxic) appeared to be unaffected. Hodson (1974) observed an increase in tolerance to zinc of rainbow trout that were acclimated to and tested at a higher temperature although the fish that died had shorter survival times than those that were acclimated to and tested at a lower temperature. He speculated that such an effect might be due to a higher metabolic rate at higher temperatures so that warm-acclimated fish may be more capable of removing zinc from sites of toxic action, or of a higher rate of synthesis of specific proteins that might otherwise be rendered inoperative. Cairns and Scheier (1957) found little difference between the toxicity of zinc to bluegills, at 15°C and 25°C. But they also found that the metal was more toxic to the fathead minnow, *Pimephales promelas*, at 25°C than at 15°C; indicating that the response of some species may be more temperature sensitive than others. More recently, Burton *et al.* (1972) showed that the survival time of bluegills in zinc was reduced with an increase in the rate of temperature change. If the decreased survival time results in a decreased tolerance to zinc, then concentrations that may not be lethal under ordinary conditions could kill fish if the environment is subjected to rapid temperature changes as could be found in bodies of water receiving heated effluents.

The dissolved oxygen concentration of water also affects the toxicity of zinc. Lloyd (1960) found that a reduction in the dissolved oxygen concentration of the test water increased

the toxicity of zinc to the rainbow trout. Similar results were obtained by Pickering (1968) for the bluegill. The former investigator also found that the effect was reduced if the trout were previously acclimated to a lower oxygen concentration. The response of fish under hypoxic conditions include increasing the rate and/or volume of ventilation and consequently the amount of toxicant exposed to the gill epithelium would increase. The observed increase in toxicity is most likely the result of adjustments made by the animal for the low oxygen levels in the medium.

Reports on the effect of pH on the toxicity of zinc appear to be contradictory. Mount (1966) observed that at any given hardness, zinc was more toxic to the fathead minnow at a higher pH (9.0) than at a lower one (5.0). The work of Cairns *et al.* (1971) on the bluegill shows that zinc was more toxic at a lower pH (6.0) than at a higher one (8.0). The former investigator suggested that a high pH could precipitate zinc from solution which then would become trapped between the gill filaments. The trapped zinc could eventually go into solution, because of the local reduction in pH resulting from carbon dioxide excretion (Lloyd and Herbert, 1960), and this dissolved zinc could then exert its toxic effect. Cairns *et al.* (1971) believe that the ionic species is the toxic form of the metal. They suggested that zinc was more toxic at the lower pH than at the higher one because under the former condition the amount of dissociation increases.

The presence of salts of other heavy metals in the test water could also affect the toxicity of zinc. The studies of Bandt, as reviewed by Doudoroff and Katz (1953), indicate that there is a synergistic effect between zinc and a) nickel, b) copper, the synergism holding for the latter only at relatively high zinc concentrations (above 1.8 mg/l zinc and 0.3 mg/l copper) (Lloyd, 1961). Synergism also exists between zinc and the cyanide ion (Doudoroff, 1956).

Although acute toxicity tests provide valuable information such as the relative toxicity of two compounds, tolerance differences among species and the effect of water chemistry on the toxicity of a compound, many authors (Crandall and Goodnight, 1962; Cairns, 1966; Alderdice, 1967; Brungs, 1969; Sprague, 1970) emphasize that these studies are not the most meaningful aspects that should be considered. The ability to survive in a medium does not necessarily indicate that the medium is satisfactory (Doudoroff, 1957). Also, concentrations that are likely to persist (Williams *et al.*, 1976) in bodies of water receiving mining and/or industrial effluents are often much lower than those used in acute toxicity studies. Consequently, the more recent studies involve sublethal levels. Tests are run for longer periods and they reflect the effects of the toxicant on parameters that are important in fish productivity: growth, fecundity, spawning behaviour, egg viability and fry survival.

Most of the available reports on the effect of zinc on growth indicate that the toxicant has an inhibitory effect.

This effect has been demonstrated in: guppies, *Poecilia reticulata*, (Crandall and Goodnight, 1962); fathead minnows (Brungs, 1969); the minnow, *Phoxinus phoxinus*, (Bengtsson, 1974a); rainbow trout (Watson and McKeown, 1976a). The only apparently conflicting report comes from the work of Pickering (1968). He reported that low zinc concentrations had a stimulatory effect on growth of the bluegill.

Zinc has also been shown to have an adverse effect on reproduction in fish. Crandall and Goodnight (1962) reported a delayed gonadal maturation in the guppy. Brungs (1969) observed that the frequency of spawning and the number of eggs laid per female were greatly reduced in the fathead minnow. The work of Saunders and Sprague (1967) indicates that the Atlantic salmon, *Salmo salar*, shows an avoidance reaction towards sublethal levels of zinc. The significance of this response is that the upstream migration of this species could be blocked, thus affecting the reproductive success of the fish population.

Crandall and Goodnight (1963) observed degenerative changes particularly in the liver, kidney and skeletal muscles of guppies following a chronic exposure to zinc. The investigators speculate that the observed gross abnormalities, such as inhibited growth, delayed sexual maturation and decreased fecundity, might be the secondary effects of stress produced directly or indirectly by the toxicant. The finding of Watson and McKeown (1976b) that the activity of Δ5-3β hydroxysteriod dehydrogenase enzyme (in the interrenal tissue),

an enzyme associated with the syntheses of corticosteroids, increases during chronic exposure of rainbow trout to zinc indicates that exposed fish are under stress.

My investigation was conducted to determine the acute and chronic toxicity of zinc to the guppy, *Poecilia reticulata*, formerly known as *Lebistes reticulatus* (see Rosen and Bailey, 1963). The guppy was chosen as the experimental animal because it can be maintained easily and breeds in the laboratory. It has a short reproductive cycle, thus making reproductive studies possible. There is a marked sexual dimorphism and one can therefore pair the fish without erring. The mode of reproduction is viviparous (Scrimshaw, 1945; Rosenthal, 1952) and consequently, the tolerance of newly born fish to zinc, following prenatal exposure to zinc, can be studied.

Estimates of 'safe' levels are usually made by multiplying LC50 values, which are determined for adults, by application factors which range from 0.01 to 0.4 (Sprague, 1971). In order to yield more reliable toxicity data, data that will not underprotect, it is necessary to base figures on the tolerance of the most susceptible stage of the life history of the fish. Information in the literature on the variation of tolerance with life history is very scanty. The present investigation will show how the tolerance of the guppy to zinc varies with the life history by comparing the tolerance of adult and immature fish.

Another aspect of this study deals with acclimation. Pickering and Vigor (1965) observed that fathead minnow fry

exposed to zinc at the egg and embryo stages were more tolerant than previously unexposed ones. Also, Sinley *et al.* (1974) showed that rainbow trout that were similarly exposed were more tolerant than those developing from non-acclimated eggs. In these previous studies, the eggs from which the more tolerant fish developed were in direct contact with the test solution. The use of a viviparous fish makes it possible to test if positive acclimation is possible in spite of the fact that the developing fish was not in direct contact with the toxicant. The tolerance of young produced by exposed females is compared to that of young from unexposed females.

The chronic tests investigate the effect of the toxicant on reproductive success and motor activity. Studies on the effect of zinc on fish activity (movement) are few and have been carried out because of the need for environmental biologists to be able to predict zinc toxicity (in the absence of fish kills). These studies (Waller and Cairns, 1972; Cairns *et al.*, 1973) are qualitative in the sense that they measure changes in movement patterns. The quantitative aspect of movement is important in the life of a fish, particularly during courtship; in events of escaping from predators and unfavourable conditions; and in food procurement. Since female guppies that are more frequently inseminated appear to produce more young in the brood than those that are not (Breder and Coates, 1932), the effect of the toxicant on courtship behaviour was also investigated.

MATERIALS AND METHODS

Source and Maintenance of Fish

Test fish were purchased locally from two commercial dealers. At the shops, fish were placed in plastic bags containing oxygen-enriched dechlorinated city tap water and transported to the laboratory. To guard against the loss of too many fish in event of disease outbreak, a maximum of 300 fish was brought to the laboratory at the same time and held in five 32-litre tanks. The holding water (and dilution water used in all experiments) was distilled water to which sodium chloride, potassium bicarbonate and calcium chloride were added in the following respective amounts: 0.03 g/l, 0.04 g/l and 0.1 g/l. The holding system was static and tanks were cleaned daily to remove any dead fish, excess food and faeces. About half of the water volume in the tanks was renewed daily. Tanks were aerated, by means of airstones placed in filters containing charcoal and glass wool, to maintain adequate oxygen levels and to provide a gentle current. Temperature in the tanks was maintained at 23-26°C, with aquarium heaters. Illumination, by means of cool white fluorescent lights (30 W), provided a 12-hour photoperiod during the holding period and for all experiments.

Fish remained in the holding tanks for a minimum period of three weeks. During this time, fish were fed twice daily from Monday to Friday on the commercial dry food preparation 'Tetramin'. This food preparation was also fed to fish in all experiments. To retard fungal growth, Nox-ich (a Weco

product) with malachite green (0.75%) as the active ingredient, was added to the tanks at the rate of one drop per four litres of holding water for three consecutive days at the end of the second week of holding. When holding periods were longer than three weeks, the fungicide was added a week before the fish were tested.

Immature fish used in the repeat of the second 10-day LC50 determination and fish used in the sublethal experiments and the acute tests in Experiment 3 were raised in the laboratory.

Test Conditions and Water Analyses

Test tanks were gently aerated to maintain adequate oxygen levels. Dissolved oxygen measurements were made using the azide modification of the Winkler method. No attempt was made to control pH but the values in test tanks were recorded using a Fisher 520 digital pH meter. Temperature was maintained at 25.5°C by placing the tanks in water tables having temperature controlled by a YSI thermostatic control connected to heater elements. This temperature was chosen because it is within the normal temperature range of the guppy (Gibson, 1954). Total hardness was determined by the EDTA titrimetric method and total alkalinity by the mixed bromocresol green-methyl red indicator method (APHA-AWWA-WPCF, 1975).

Preparation and Determination of Zinc

An acidified stock solution (1 ml reagent grade nitric acid per litre of solution) of 6,000 mg/l zinc sulphate ($ZnSO_4 \cdot 7H_2O$) was prepared using reagent grade salt. For

each test solution, the appropriate volume of zinc stock solution was pipetted into a 6-litre conical flask and dilution water was added up to the 6-litre mark. The test solution was then thoroughly stirred on a Fisher Thermomix using a magnetic bar.

To prevent the loss of significant amounts of zinc, test solutions were partially renewed by siphoning out half of the volume of solution in each tank and replacing it with fresh solution. Solutions were only partially renewed to minimize handling of fish.

Zinc determinations were made using a Jarrell-Ash (Fisher) Atomic Absorption Spectrophotometer. Aspiration of standards and samples was direct (i.e. no extraction). Because the dilution water was the matrix used in all zinc determinations, control tanks were assigned zero zinc concentration values.

Experiment 1. Determination of LC50

The test system was static and glass tanks of the following outside dimensions: 21 (l) x 25 (w) x 20 (h) cm were used. These held 8 litres of test solution each. Each tank had a glass cover set over it to reduce evaporation, keep away dirt and prevent fish loss through jumping. Test tanks in other experiments also had glass lids. Dissolved oxygen, zinc and pH measurements were made daily.

Preliminary tests lasting 7 days and involving 10 fish per tank and concentrations that ranged from 1 to 10 mg/l zinc, were carried out on immature and adult fish to determine the range of concentrations that would be used in the LC50 deter-

minations. From the preliminary test results, the lowest concentration chosen for the LC50 determinations was the highest concentration at which no mortality occurred. The highest concentration used was the lowest that caused less than 100 per cent mortality. For adult fish, the concentrations used were 2.73, 3.73, 4.23, 5.23, 6.23 and 6.73 mg/l zinc. For immature fish, these were 1.23, 1.73, 2.63, 3.73, 4.23 and 4.73 mg/l zinc.

Three sets (A,B,C) of LC50 determinations were made for both adult and immature fish and the procedures mentioned below were followed in each case.

A day prior to beginning the experiment, test tanks were washed with detergent, rinsed with distilled water and wiped with 0.1N solution of ethylene-tetraacetic acid, washed again with detergent and rinsed several times with distilled water. These steps were to ensure that no zinc was present in the tanks. Fish to be used were selected (from tanks in which mortality during the previous week did not exceed 10 per cent): fish were anaesthetized with 0.1 per cent ethyl m-aminobenzoate methanesulfonate (MS 222) solution and the standard lengths (from the tip of the snout to the end of the vertebral column) were measured using vernier calipers. Adult fish that were more than 30 mm, or less than 18 mm (= small males) in length were not used. For immature fish, the respective limits were 16.5 and 7.5 mm. The fish were then weighed individually and the upper and lower limits were set at 0.7 g and 0.15 g for adult fish and 0.09 g and 0.01 g

for immature fish. The adult fish weighed 0.49±0.12 g (mean ± one standard error) and the standard length was 23.44±0.15 mm. For immature fish, these values were 0.03±0.001 g and 12.43±0.12 mm respectively. The fish of each size group were then randomly assigned to one of 14 tanks ({6 experimental + 1 control} x 2), which at this time contained dilution water, by means of numbered slips of paper drawn from a container. Each tank held 10 fish.

To begin the tests, the ten fish were removed from each of the tanks and the tanks were emptied. The test solutions were then poured into the tanks from the flasks after first rinsing each with some of the appropriate test solution. Control tanks contained dilution water only. Fish were then returned to their tanks. Each of the six zinc concentrations was tested in duplicate at the same time, so that the measured zinc concentration and the observed per cent mortality at each concentration were the averages of two determinations each.

Fish were not fed during the 24-hour period preceding the tests. They were fed on day 5 before the test solutions were partially renewed. Tanks were checked for dead fish every 4 hours from 8 a.m. to 12 midnight. Death was assumed to have occurred if respiratory and fin movements ceased and the fish did not respond when pricked with a mounted pin.

Statistical Method

The 10-day LC50 values were estimated for both adult and immature fish as outlined by Litchfield and Wilcoxon (1949): per cent mortality (probit scale) was plotted against the measured zinc concentration (log scale) at which it occurred. A straight line was fitted to the points by eye and its goodness of fit was tested by $(\text{chi})^2$ analysis. The LC50 values were then interpolated from the graphs. Probit regression lines were calculated (see Finney, 1947) for adult and immature fish by combining the 3 sets of data (A,B,C) in each case.

The significance of the difference in LC50 values estimated for adult and immature fish was evaluated by a t-test.

In this and all other experiments, significance was accepted at $P \leq 0.05$.

Experiment 2. Effect of chronic exposure to zinc on reproduction

Brood size in this fish is related to the size of the female, large females usually producing larger brood sizes (Turner, 1937; Purser, 1938). Also, the size of the previous brood may affect the size of the next brood; the number of young increasing to a maximum and then decreasing to a minimum (Rosenthal, 1952). Age could therefore be another factor to be considered. To decrease variability from one female to another it was necessary to use virgin females of approximately the same size and age.

These females (not necessarily siblings) were obtained in the following manner: gravid females were removed from the holding tanks and placed in breeding traps that were suspended in breeding tanks. This was done from the beginning of March to the end of the first week of April, 1977. Each female was returned to the holding tanks as soon as the brood was delivered. The breeding tanks were heated by means of aquarium heaters and the temperature fluctuated between 23 and 28°C. The fry were fed twice daily from the time they were a week old. Daily observations were made for individuals (males) that were just beginning to show an elongation of the anal fin. These were removed as soon as they were found. The breeding tanks gradually became all-female tanks. The success of the method was reflected by the fact that there were no fry found in the all-female tanks throughout the growth period nor were females ever observed to be gravid.

The test system was static and 20 small tanks (5 tanks for each of the three zinc concentrations and the five controls) measuring 15(h) x 20(l) x 20(w) cm and holding 4.5 litres of test solution were used. At the beginning of the experiment, the tanks were washed with detergent and sterilized by rinsing with 70 per cent ethyl alcohol. The tanks were then rinsed twice again with distilled water. This procedure was repeated halfway through the experiment. Virgin females of approximately the same length were assigned to each of the 20 tanks (one female per tank) and the tanks were assigned randomly to four groups of five. Individuals in each group were anaesthetized with a

0.1 per cent MS 222 solution and the standard lengths of individual fish were recorded (Appendix 4). The data were analyzed by an Anova test to ensure that significant size differences did not exist between groups. The females were then paired (August 18) with selected males from the holding tanks. The selection was done on the basis of courtship display. Males showing less than 9 displays per minute were not used.

The test concentrations (nominal values), 1.93; 0.97 and 0.48 mg/l zinc, respectively represent 0.4, 0.2 and 0.1 of the 10-day LC50 determined for adults. These concentrations were assigned to three groups of fish and dilution water served as control (the fourth group). Zinc was introduced into the test tanks a day after the fish were paired. Test solutions were partially renewed on Mondays and Thursdays and zinc concentrations were determined using acidified 7-day composite samples. Dissolved oxygen was determined monthly; pH weekly; total hardness and total alkalinity every other week. Fish were fed twice daily for five days (Monday-Friday) a week.

As the female in a test tank became noticeably pregnant, the pair was put into a breeding cage suspended in the test tank. Because many test females did not show a typical gravid appearance, all pairs were isolated in the breeding cage after 20 days of a particular interval of brood production. The

pair remained in the cage until the brood was delivered, then the young and the breeding cage were removed. The breeding cages were made of nylon netting with 3 mm appertures. Isolation of pairs was necessary since guppies are cannibalistic and parents would chase and eat their young.

The experiment was run for four months during which time four broods were produced. Observations for brood production were made at least twice daily, in the morning and in the late evening. While courtship behaviour and motor activity studies lasted, an additional observation was made in the afternoon. The brood production record of each female was kept. It comprised the number of young in each brood (=brood size) and the date on which the brood was discovered (the brood is usually delivered within an hour). From the latter, intervals between broods were calculated. As soon as the brood was discovered, the juveniles were removed and anaesthetized with 0.1 per cent solution of urethane (ethyl carbamate) and the standard lengths were obtained by measuring with vernier calipers under a binocular microscope. A maximum of 10 fry per brood were used in the size determinations.

Statistical Methods

The significance of differences between groups with respect to brood size, total number of young per female, intervals between broods and size of young at birth was tested by Anova. When the difference was significant, means were

compared using the Duncan's New Multiple Range test. The effect of time in a zinc solution on the variables was determined by within group comparisons (using the above tests of significance).

Experiment 3. Effect of prenatal exposure to zinc on the acute toxicity of zinc to young guppies

Fish used in this experiment were produced by the exposed (3 zinc concentrations) and unexposed (control) females in Experiment 2. Because of the small brood size of exposed females, young used for length measurements in Experiment 2 were used in some of these tests. This was done only when recovery, from the anaesthesia, in the zinc concentration at which they were prenatally exposed was 100 per cent. A maximum of ten and a minimum of five young were used in each test. When five fish were used, there was always a repeat when fish became available and both sets of data were combined so that a total of ten fish were involved in each test.

The zinc concentration used in this test was 6 mg/l zinc. Test apparatus was static; test chambers were four-litre jars that held 3 litres of test solution. Because 100 per cent mortality occurred within 24 hours in all cases, test solutions were not renewed. Fish were not fed during the experiment.

To begin the tests, the test solution was introduced into the test chambers and the appropriate number of young fish was removed by a small hand net from the tanks in which they were born and put into the acutely toxic solution. Five tests were run per group of prenatal exposure. Because

broods produced late at night were not detected and tested until the next morning, a second control test was run. This involved exposing young produced between 8 p.m. and 11 p.m. by control mothers to 0.97 mg/l zinc (one of the three sublethal concentrations of prenatal exposure) for 12 hours before being tested in 6 mg/l zinc.

In all tests, individual time to death was recorded. Fish were assumed dead when movement ceased and the young fish did not respond when pricked with a mounted pin.

Statistical Method

The data were treated in the same manner as those in Experiment 1. The difference was that the log-probit plot was between cumulative per cent mortality and survival time. The interpolated values were LT50s, the median survival times (hours).

Experiment 4. Effect of chronic exposure to zinc (during Experiment 2) on motor activity and the intensity of courtship

A description of the courtship pattern is presented before attempting to quantify courtship in this fish. The most easily observed feature of the courtship behaviour is the persistent manner in which the males pursue the females. During the chase, the males often thrust at the genital region of the females with the gonopodium (a modified anal fin which serves as an intromittent organ). The movement of the gonopodium is referred to as "Gonopodial swinging'. During this act, the gonopodium is swung laterally and forward

and there is continued rotation until the tip of the gonopodium which points posteriorly during normal swimming now points anteriorly (Clark and Aronson, 1951). Other parameters of the courtship pattern include the 'Dorsal fin erection' and the 'Sigmoid reaction'. In the former, the dorsal fin is spread and in the latter, the body is thrown into an S-shape, the body quivers, the caudal fin is spread and there is rapid movement backwards and forwards over a short distance (Liley, 1966). The arching of the body may be maintained for 3-5 seconds usually, but extreme values of 15 seconds are possible.

Sperm transfer involves copulation, an act which may last for less than a fraction of a second (Clark and Aronson, 1951) and is therefore less commonly observed than the other features. Both fish make contact momentarily and wheel around in small circles before swimming apart.

The pattern described above refers to the male's behaviour. The displays will occur in the absence of females (Breder and Coates, 1935; Warren, 1973). The presence of females, however, increases the frequency of display (Warren, 1973). The females have a less easily defined courtship behaviour pattern. The behaviour of the females towards displaying males has been categorized as 'passive' (Breder and Coates, 1935; Clark and Aronson, 1951) in the sense that they do not seem to show an active response to male courtship. Assumption of a stationary position by the female appears to be a sign of receptivity (Clark and Aronson, 1951). More recently, Liley (1966) described a more active response pattern for females. It

involves gliding towards the displaying male, halting and raising the tail and head slightly.

This experiment was run simultaneously with Experiment 2 and both involved the same fish and the same test solutions. The front side of each water table was replaced with a sheet of plexiglass thus making observations from the side of the tanks possible.

A. Motor Activity

A grid comprising vertical and horizontal lines that were 5 cm and 3.5 cm apart respectively, was drawn on the observation face of each test tank using a diamond-tipped pencil. Thrice every week for 12 weeks, activity of the female was measured for 5 minutes by counting the number of times the fish crossed the vertical (=horizontal movement) and the horizontal lines (=vertical movement). The males were removed one hour before the observations and were returned after the observations. Activity was measured from 12 noon (three hours after fish were fed) to 2 p.m.

B. Courtship Behaviour

Although a more thorough study would have been one that incorporated male and female responses, it was difficult even after several observations to accurately recognize the female response if and when it occurred. The study was therefore based on male responses only. Since mating would, to a certain extent, include female responses, gonopodial swings recorded were only those in which contact was actually or nearly made with the genital area of the female. Some of

the gonopodial swings might therefore represent incidents of mating.

Observations on courtship behaviour were made on days on which motor activity was not measured, beginning at 12 noon. The length of the observation period of each pair of fish was 3 minutes and three observations were made per week. Courtship intensity was determined by counting the number of times the following displays occurred:

a) dorsal fin erection
b) Sigmoid reaction
c) gonopodial swinging

In both female motor activity and male courtship behaviour measurements, the order of observation was randomized.

Statistical Methods

In both cases, the time scale was divided into four 3-week intervals. The same tests of significance used in Experiment 2 were used to evaluate between and within group differences.

RESULTS

Experiment 1. LC50 Determination

Results of the routine water analyses show that the range of pH values (6.8 to 8.0) was reasonably narrow and the highest pH values were recorded in control tanks; alkalinity and total hardness showed fairly wide variations (16 to 24 mg/l; 80 to 120 mg/l as $CaCO_3$ respectively); oxygen levels were close to saturating values; range of temperature values did not exceed 1°C (Appendix 1). Aerating the tanks had the additional advantage of providing a gentle current which aids the mixing of ions and thus makes for more uniform test solution concentrations within the tanks.

The difference between measured and nominal zinc concentrations was not appreciable (Appendix 2).

Some of the adult fish used were almost twice as long as the shortest ones. This could not be avoided partly because of the large number of fish used in the study and partly because of the sexual dimorphism existing in the adult fish with respect to size.

There was an increase in mortality with increase in zinc concentration except in two cases (Adult A, 6.02 and 5.09 mg/l zinc; Immature B, 4.67 and 4.26 mg/l zinc) where the higher concentration caused the same per cent mortality as the next lower concentration (Appendix 2). Plots of cumulative per cent mortality versus time to death (Figures 1 and 2) show that apart from the increase in mortality with increase in concentration, the higher concentrations also

decreased survival time. Thus adult fish that died in 6.73 mg/l zinc did so between the first and sixth days whereas fish in the lower concentrations survived longer (Figure 1). A similar trend can be seen for immature fish (Figure 2).

The three, 10-day LC50 values determined (Litchfield and Wilcoxon method) for adult and immature fish are presented in table 1. The three sets of mortality data (Appendix 2) were then combined for adults (Figure 3) and immatures (Figure 4). The LC50 values obtained by the Finney method were close to the respective averages of the three sets of determinations by the Litchfield and Wilcoxon method (Table 1). From the former values, adults were estimated to be 1.8 times as tolerant as immatures. This tolerance difference was significant.

Experiment 2. Effect of zinc on reproduction

Properties of test water in the sublethal experiments (reproductive, motor activity and courtship behaviour) did not vary much during the study (Appendix 3). Zinc concentrations decreased in all tanks and the mean values (0.36, 0.88 and 1.70 mg/l zinc) were used to characterize the tests.

During the course of the experiment, two female fish, one in 0.36 mg/l zinc and the other in 1.70 mg/l zinc, died in the sixth and seventh weeks respectively. Both fish could not be replaced because of a fungal infection among virgin females in the holding tank. This infection was not present at the start of the experiment and both females that died did not have the infection.

Figure 1. Mortality of adult guppies in three concentrations of zinc during one of the LC50 (A) determinations.

Nominal conc. (mg/l zinc)	Test[1]	Symbol
5.23	1	■
5.23	2	□
6.23	1	▲
6.23	2	△
6.73	1	●
6.73	2	○

[1] 1 and 2 are duplicates.

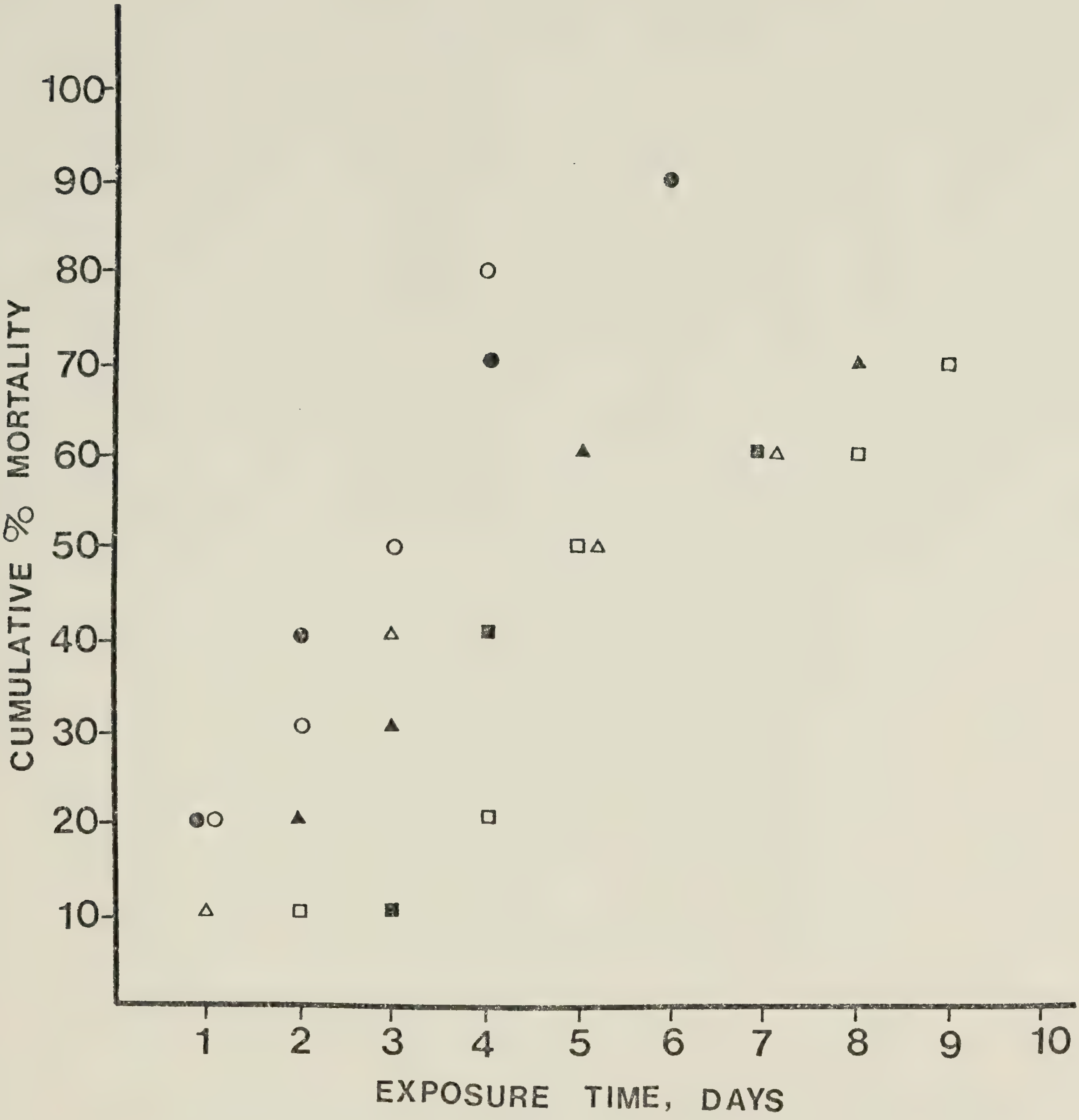

CUMULATIVE % MORTALITY
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
EXPOSURE TIME, DAYS

Figure 2. Mortality of immature guppies in three concentrations of zinc during one of the LC50 (A) determinations.

Nominal conc. (mg/l zinc)	Test[1]	Symbol
3.73	1	■
3.73	2	□
4.23	1	▲
4.23	2	△
4.73	1	●
4.73	2	○

[1] 1 and 2 are duplicates.

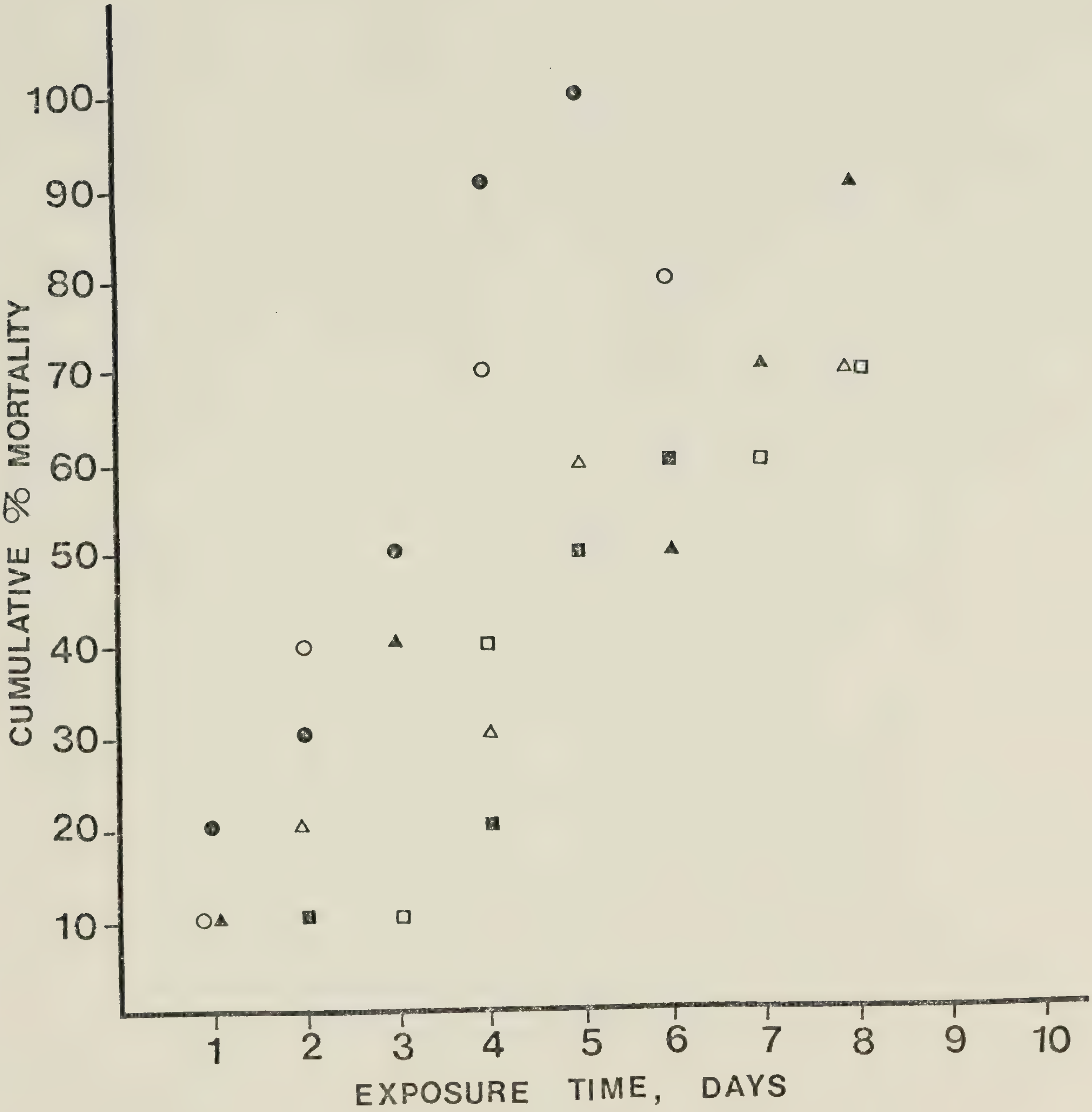

CUMULATIVE % MORTALITY
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
EXPOSURE TIME, DAYS

Figure 3. Probit regression line for estimating the 10-day LC50 of adult guppies (data in Appendix 2).

Symbol	LC50 determination
○	A
●	B
▲	C

Zinc concentrations are measured values.

In the equation,

y = % mortality in probits

x = log of zinc concentration.

The curved lines represent 95% confidence bands for the line.

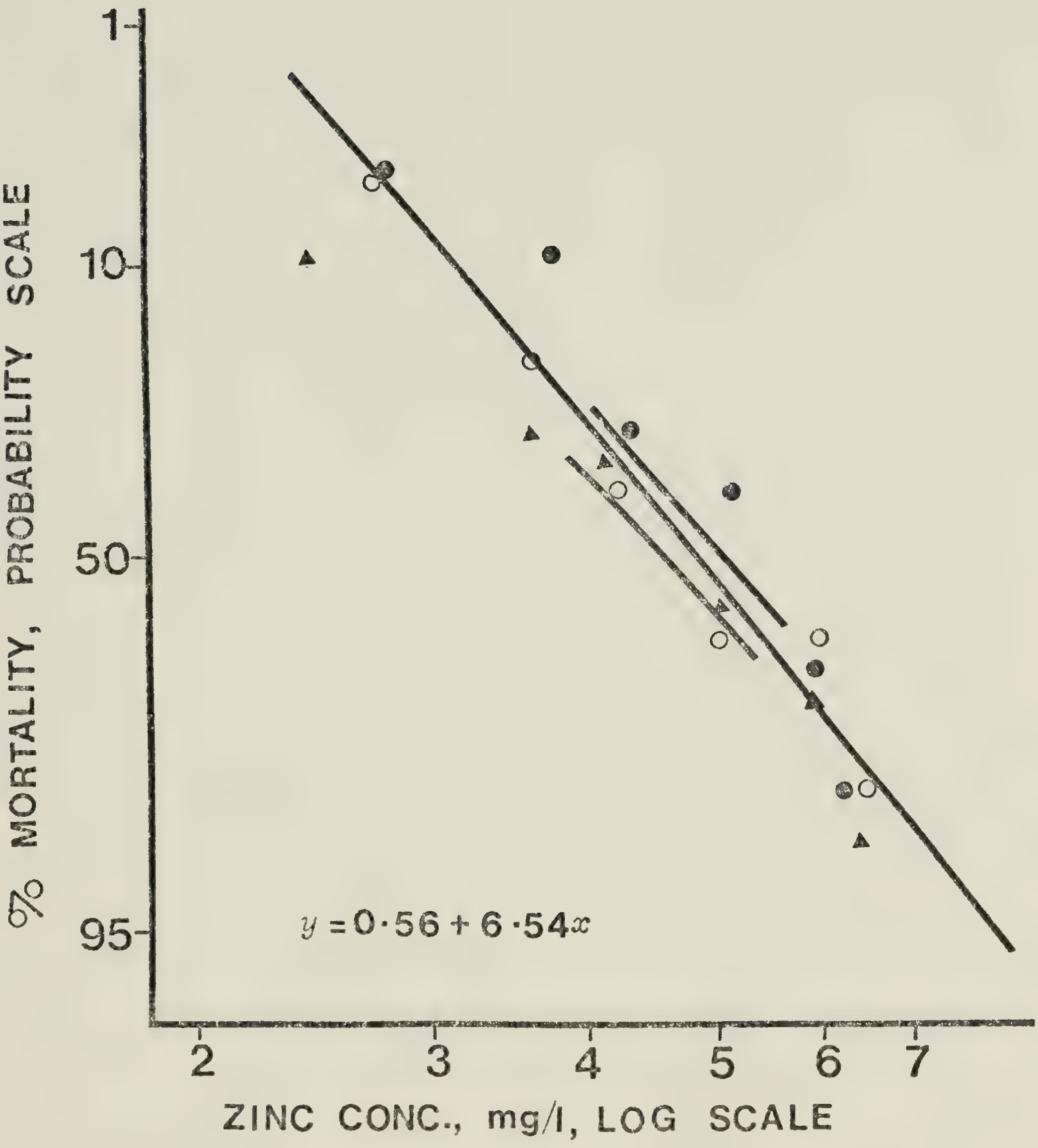

% MORTALITY, PROBABILITY SCALE
1
10
50
95
$y = 0{\cdot}56 + 6{\cdot}54x$
2
3
4
5
6
7
ZINC CONC., mg/l, LOG SCALE

Figure 4. Probit regression line for estimating the 10-day LC50 of immature guppies (data in Appendix 2).

Symbol	LC50 determination
○	A
●	B
▲	C

Zinc concentrations are measured values.

In the equation,

y = % mortality in probits

x = log of zinc concentration

The curved lines represent 95% confidence bands for the line.

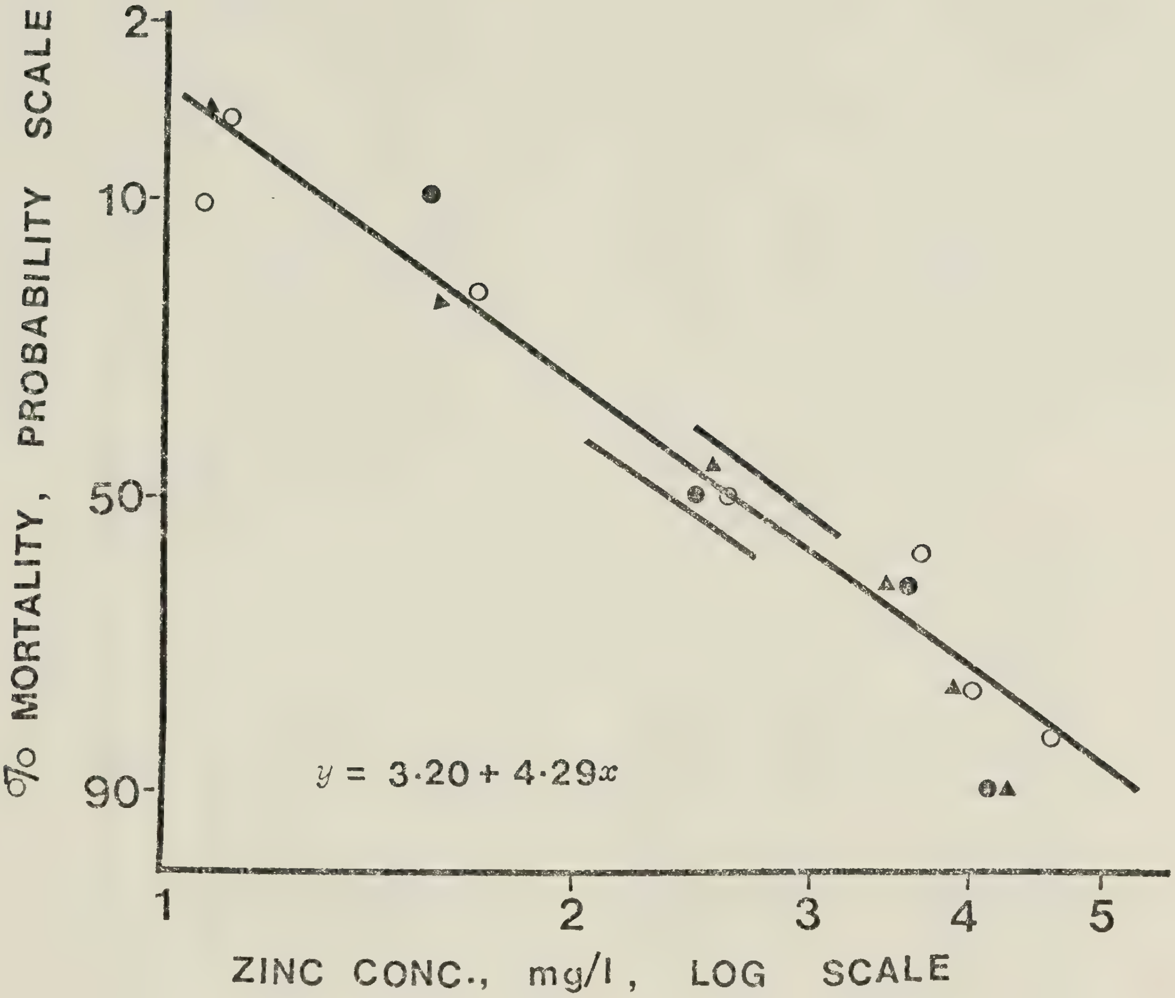

% MORTALITY, PROBABILITY SCALE
2
10
50
90
1
2
3
4
5
ZINC CONC., mg/l, LOG SCALE
$y = 3{\cdot}20 + 4{\cdot}29x$

TABLE 1. 10-day LC50 (mg/l zinc) determined for adult and immature fish. 1977

	Test (date)	LC50[1]	$\bar{X}$	LC50[2] (Confidence limits)
Immature	A(June 8-18)	2.61		
	B(July 1-11)	2.68		
	C(July 28-Aug. 7)	2.66	2.65	2.63 (2.40-2.88)
Adult	A(May 25-June 4)	4.80		
	B(June 20-30)	5.20		
	C(July 12-22)	4.50	4.83	4.79 (4.57-5.01)

[1] LC50 determined by Litchfield and Wilcoxon method.

[2] LC50 determined by Finney's method.

Zinc had no effect on the intervals between broods: between group differences at any parturition period were not significant and although the intervals were shortest between pairing and the production of the first brood (Figure 5), within group differences were also not significant.

To a large extent, the total number of young produced per female through the four parturition periods decreased with increase in zinc concentration (Figure 6), exposure to 0.36, 0.88 and 1.70 mg/l zinc causing 24, 58 and 50 per cent reduction respectively. This decrease was not caused by a decreased frequency of brood production of exposed fish since intervals between broods were not significantly different. The results indicate that the effect of the toxicant is on the brood size.

Over the four parturition periods (Figure 6), brood sizes of females in 0.88 and 1.70 mg/l zinc were significantly lower than those of control females; females in 0.36 mg/l zinc had significantly larger brood sizes than those in 0.88 mg/l zinc. Brood sizes in 1.70 mg/l zinc were larger than in 0.88 mg/l zinc but the difference was not significant. Brood sizes in 0.36 mg/l were larger than in 1.70 mg/l zinc but the difference was not significant. Brood sizes of control females were larger than those produced by females in 0.36 mg/l zinc but the difference was not significant.

The length of the exposure period affected the brood sizes (Figure 7). After the first parturition period, the brood sizes of females in 0.88 and 1.70 mg/l zinc showed a

Figure 5. Effect of chronic exposure to zinc, through four parturition periods, on the interval between broods in the female guppy.

Values are $\bar{X}$ ± 1SE (details in Appendix 5)

Concentrations are measured values.

A = 0 mg/l zinc

B = 0.36 mg/l zinc

C = 0.88 mg/l zinc

D = 1.70 mg/l zinc

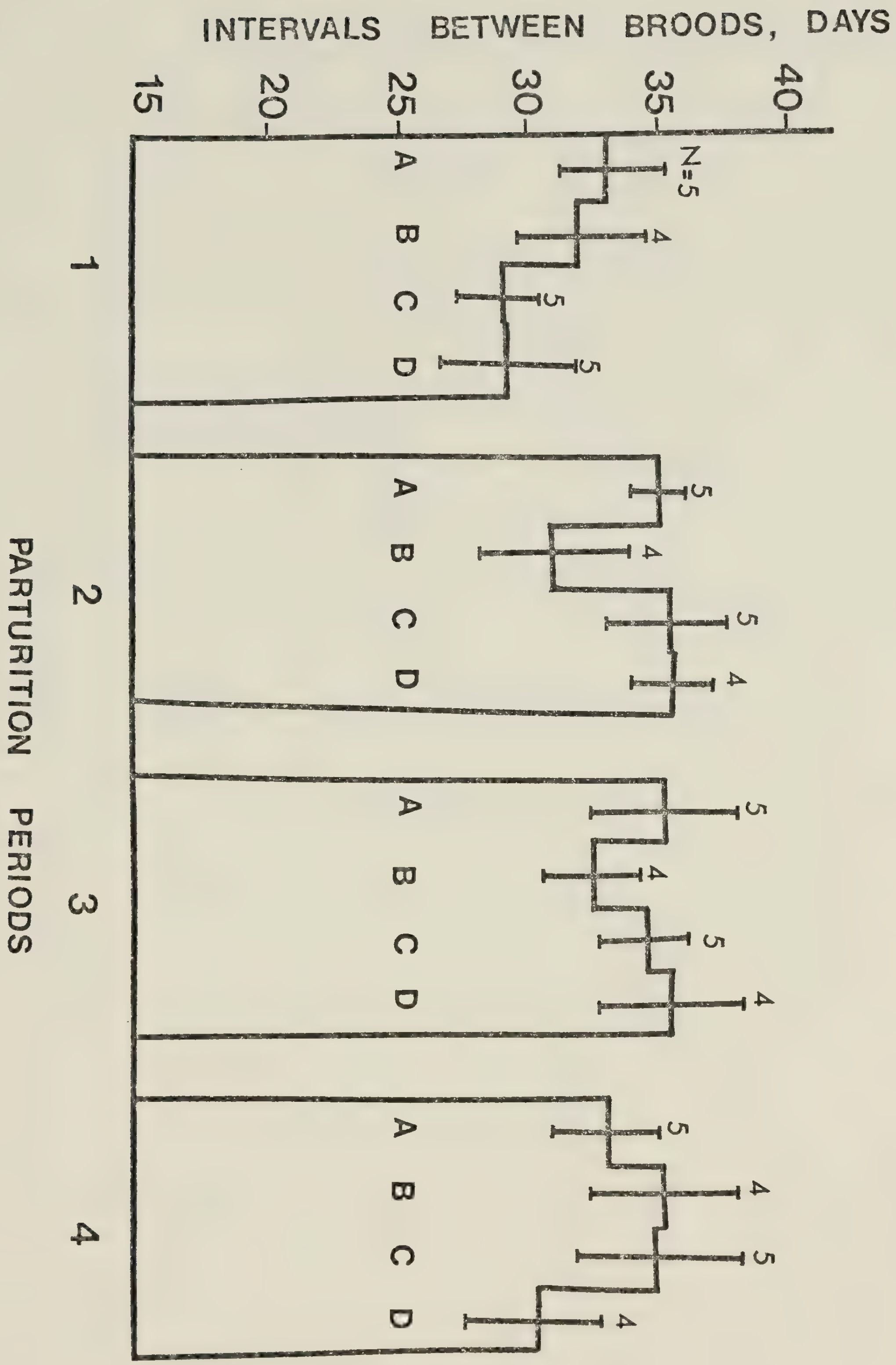

INTERVALS BETWEEN BROODS, DAYS
15
20
25
30
35
40
N=5
4
5
5
5
4
5
4
5
4
5
4
5
4
5
4
A
B
C
D
1
2
3
4
PARTURITION PERIODS

Figure 6. Effect of chronic exposure to zinc on

a) the total number of young produced per female guppy (solid lines)

b) the number of young per brood (dash line)

in four parturition periods.

Values are $\bar{X} \pm 1SE$ (details in Appendix 6).

Concentrations are measured values.

A = 0 mg/l zinc

B = 0.36 mg/l zinc

C = 0.88 mg/l zinc

D = 1.70 mg/l zinc

Between group comparison

a) $$\begin{array}{cccc} C & D & B & A \\ \cline{3-4} & & & \\ \cline{1-3} \end{array}$$

b) $$\begin{array}{cccc} C & D & B & A \\ \cline{3-4} & & & \\ \cline{2-3} & & & \\ \cline{1-2} \end{array}$$

Groups not underscored by the same line have significantly different means.

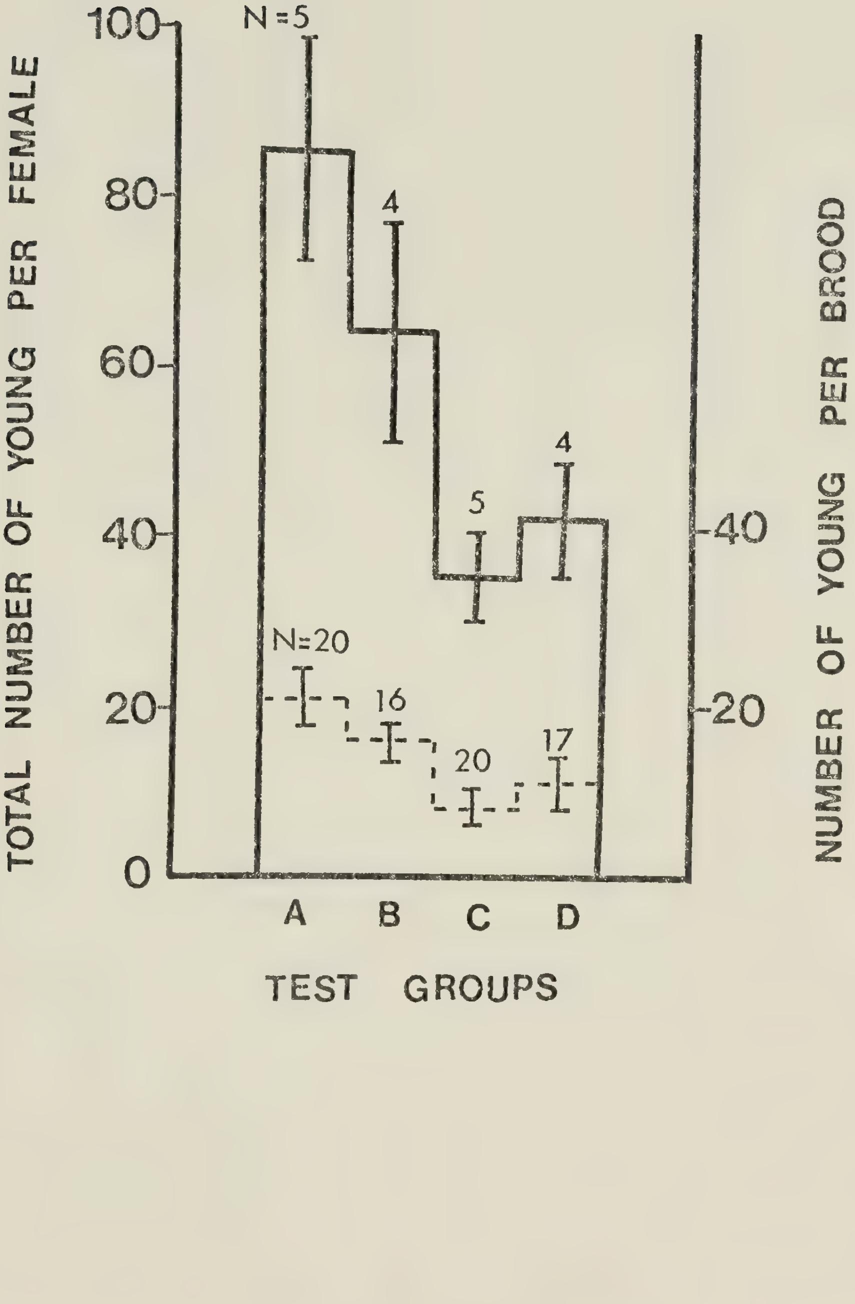

TOTAL NUMBER OF YOUNG PER FEMALE
100
80
60
40
20
0
NUMBER OF YOUNG PER BROOD
40
20
N=5
4
5
4
N=20
16
20
17
A
B
C
D
TEST GROUPS

Figure 7. Changes in the brood size of the female guppy during chronic exposure to zinc through four parturition periods.

Values are $\bar{X}$ ± 1SE (details in Appendix 6)

Concentrations are measured values.

A = 0 mg/l zinc
B = 0.36 mg/l zinc
C = 0.88 mg/l zinc
D = 1.70 mg/l zinc

Between group comparison during each parturition period:

1st $\underline{B\quad A\quad C\quad D}$

2nd $\underline{D\quad C}\quad\underline{B\quad A}$

3rd $\begin{array}{cccc} C & D & B & A \\ \cline{3-4} \\[-1ex] \cline{1-3} \end{array}$

4th $\begin{array}{cccc} C & D & B & A \\ \cline{3-4} \\[-1ex] \cline{1-3} \end{array}$

Within group comparison: (numbers refer to the parturition periods and the order does not necessarily represent increasing values).

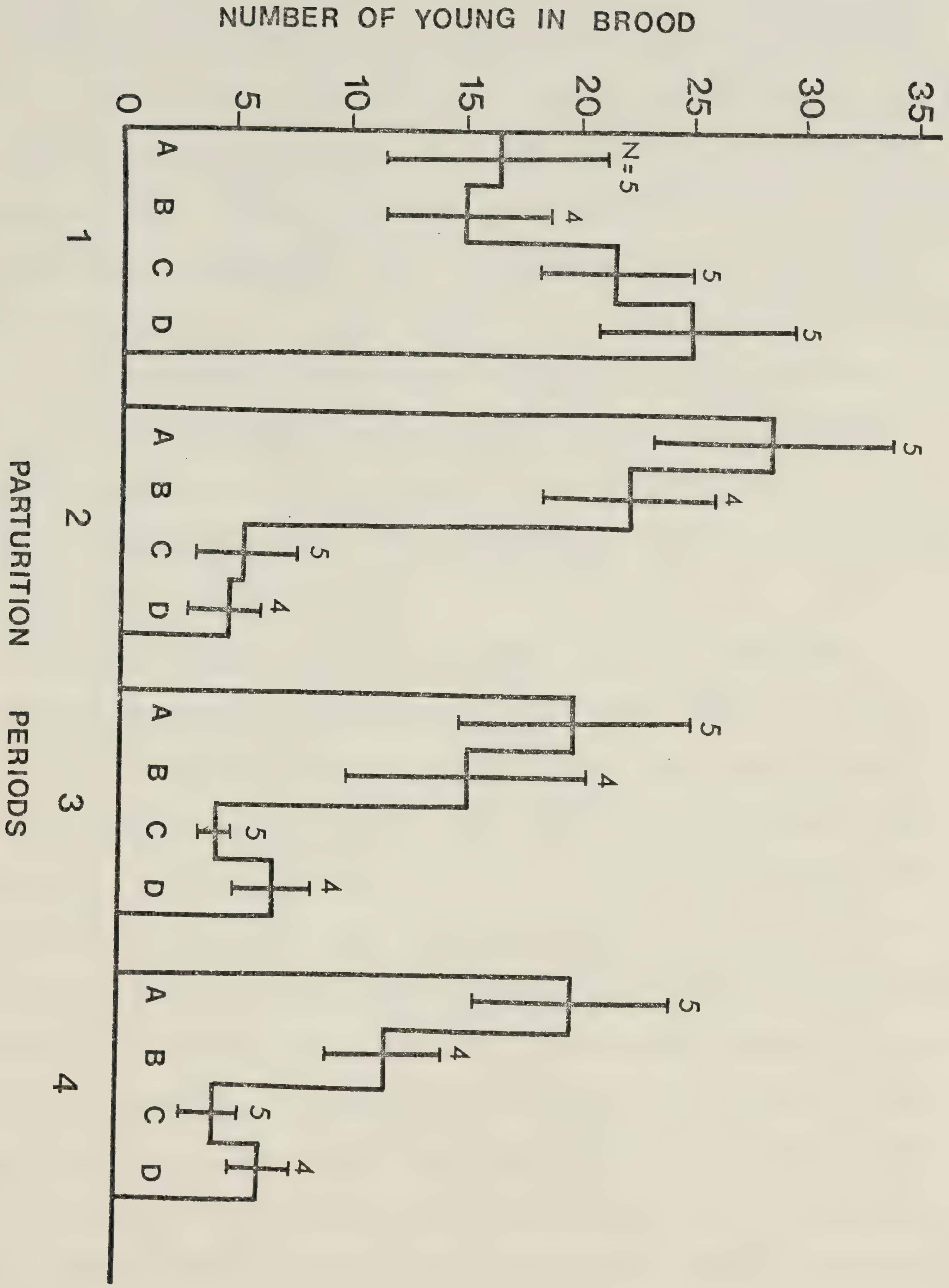
A $\underline{1\quad 2\quad 3\quad 4}$

B $\underline{1\quad 2\quad 3\quad 4}$

C $1\quad\underline{2\quad 3\quad 4}$

D $1\quad\underline{2\quad 3\quad 4}$

Groups not underscored by the same line have significantly different means.


NUMBER OF YOUNG IN BROOD
0
5
10
15
20
25
30
35
N=5
4
5
5
5
4
5
4
5
4
5
4
5
4
5
4
A
B
C
D
A
B
C
D
A
B
C
D
A
B
C
D
1
2
3
4
PARTURITION PERIODS

significant decrease. Brood sizes during the second, third and fourth parturition periods were only 25%, 19% and 19% of the brood size in the first parturition period for females in 0.88 mg/l. In 1.70 mg/l zinc, these values were 18%, 27% and 25%. Females in 0.36 mg/l zinc showed an increase in the brood size during the second parturition period followed by decreases during the third and fourth parturition periods but the difference was not significant throughout the duration of the experiment. Brood size in control tanks increased during the second parturition period and decreased in the third parturition period (same brood size for 3rd and 4th parturition periods) but the differences were not significant from one period to another.

Between group comparisons during each of the four parturition periods show that in the first period, females in the two highest zinc concentrations (0.88 and 1.70 mg/l zinc) produced larger brood sizes than control females and females in 0.36 mg/l zinc. However, these differences were not significant. In the second parturition period, females in the two highest zinc concentrations produced significantly smaller brood sizes than control females and those in 0.36 mg/l zinc. In the third parturition period, brood sizes in all three zinc concentrations were not significantly different and only the brood sizes in the two highest zinc concentrations were significantly smaller than values for control females. The same trend was shown in the fourth parturition period (see Figure 7 legend).

Only live young were taken into consideration when recording the brood size. In all zinc concentrations, dead young were produced and these had the following abnormalities: they were less than 4 mm long (total length), had not absorbed the yolk completely and had not uncurled from the embryonic posture. These individuals represent 4%, 10% and 4% of the total number of young produced in 0.36, 0.88 and 1.70 mg/l zinc respectively. In 0.88 mg/l, there were three cases in which the fertilized egg had not developed into a distinguishable embryo (included as dead young). In each zinc concentration, whenever abnormal young were produced, the brood also contained live individuals. There were no apparent abnormalities among live fish produced by control females and those in zinc and no dead young were found in control tanks. Dead young (in the zinc concentrations) dropped into the test tanks from appertures of the breeding cages. Also, observations during the present investigation indicate that guppies are not likely to notice and eat young that are not moving. It is therefore unlikely that control fish had eaten their dead young.

Dead young were also excluded from the determination of the size (standard length) of fry at birth. Over the four parturition periods, females in 0.88 and 1.70 mg/l zinc produced significantly smaller young than those in 0.36 mg/l zinc and controls (Figure 8). Between group comparisons during each parturition period show that: 1) the differences mentioned above occurred in the first parturition period, 2) in the second parturition period, females in 0.88 mg/l

Figure 8. Effect of chronic exposure of female guppies to zinc on the size (standard length) of fry at birth.

Values are $\bar{X} \pm 1SE$ (details in Appendix 7).

Concentrations are measured values.

A = 0 mg/l zinc
B = 0.36 mg/l zinc
C = 0.88 mg/l zinc
D = 1.70 mg/l zinc

Between group comparison through the four parturition periods:

$\underline{D \quad C} \quad \underline{B \quad A}$

Between group comparison at each parturition period:

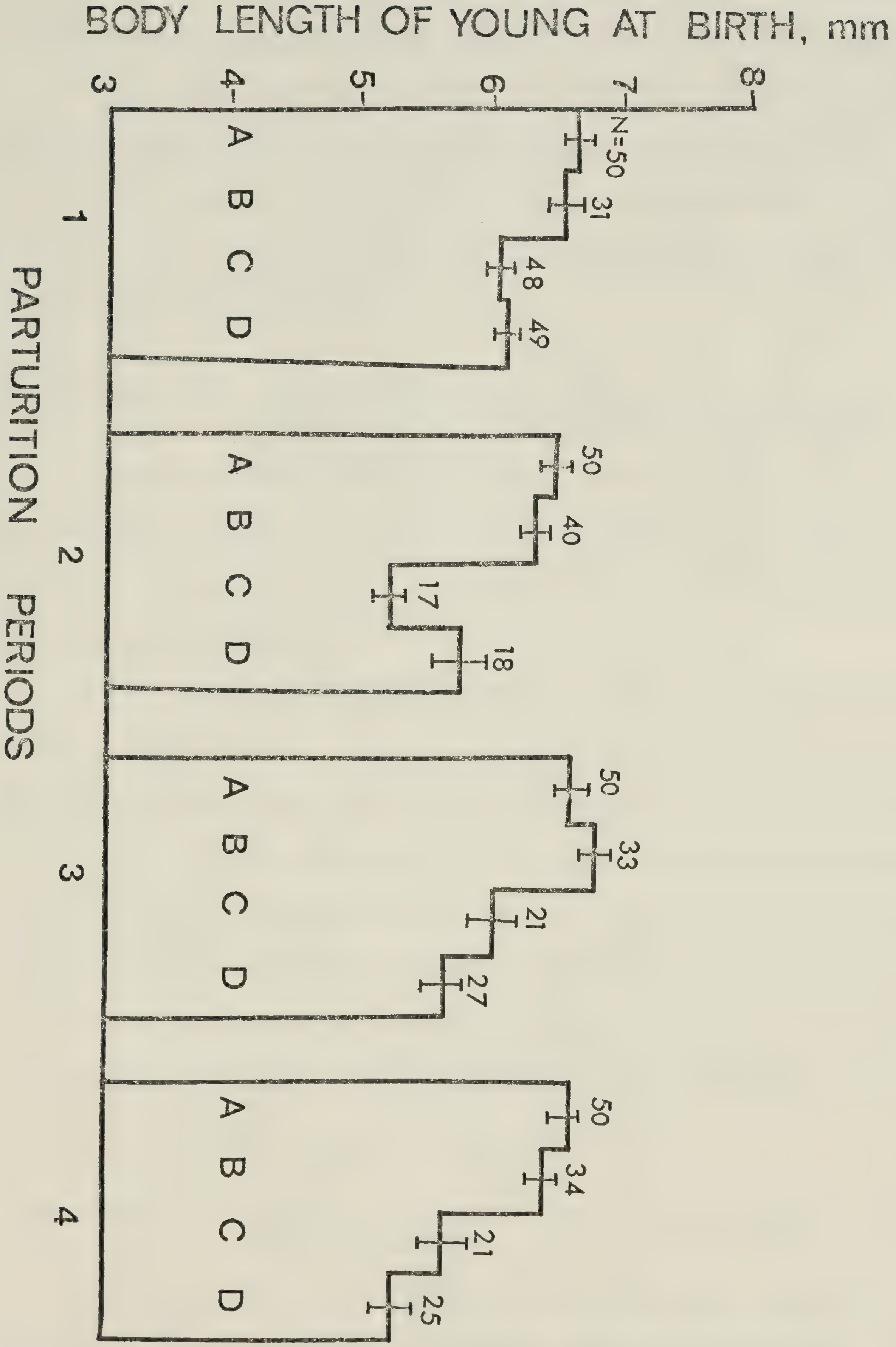
1st $\underline{C \quad D} \quad \underline{B \quad A}$

2nd $C \quad D \quad \underline{B \quad A}$

3rd $\underline{D \quad C} \quad \underline{A \quad B}$

4th $\underline{D \quad C} \quad \underline{B \quad A}$

Groups not underscored by the same line have significantly different means.


BODY LENGTH OF YOUNG AT BIRTH, mm
3
4
5
6
7
8
N=50
31
48
49
50
40
17
18
50
33
21
27
50
34
21
25
A
B
C
D
1
2
3
4
PARTURITION PERIODS

zinc produced significantly smaller sized young than those in 1.70 mg/l zinc and values obtained for either group of females were significantly smaller than those for females in 0.36 mg/l zinc and controls, 3) in the third and fourth parturition periods, the trends were similar to that in the first parturition period (see Figure 8 legend for details). Within group comparisons were not significant at any of the four parturition periods.

Experiment 3. Effect of prenatal exposure to zinc on the acute toxicity of zinc to young guppies

The calculated (Finney, 1947) median survival times (Figures 9-13) were close to the respective averages of the median survival times estimated by the Litchfield and Wilcoxon (1949) method (Table 2).

Fish that had been prenatally exposed to 0.88 mg/l zinc had a significantly longer survival time than C_1 young (controls) and young that were prenatally exposed to 1.70 mg/l zinc (Table 2). The difference in survival time between young that were prenatally exposed to 0.36 mg/l zinc, 0.88 mg/l zinc and C_2 (controls that were postnatally exposed to 0.97 mg/l zinc for 12 hours prior to acute toxicity test) was not significant nor was it between those exposed to 1.70 mg/l zinc, 0.36 mg/l zinc, C_1 and C_2 (controls).

Experiment 4. Effect of zinc on courtship behaviour and motor activity

Results of the courtship behaviour study (Figure 14) indicate that gonopodial swinging was displayed less often

Figure 9. Probit regression line for estimating the LT50 (hours) of young guppies (C_1)[1] in 6 mg/l zinc.

Test Symbol	Source of young
○	1st brood of ♀ 4
●	2nd brood of " 2
△	2nd brood of " 5
▲	3rd brood of " 4
■	4th brood of " 4

In the equation,

y = % mortality in probits

x = log of time (hours)

[1] Control fish.

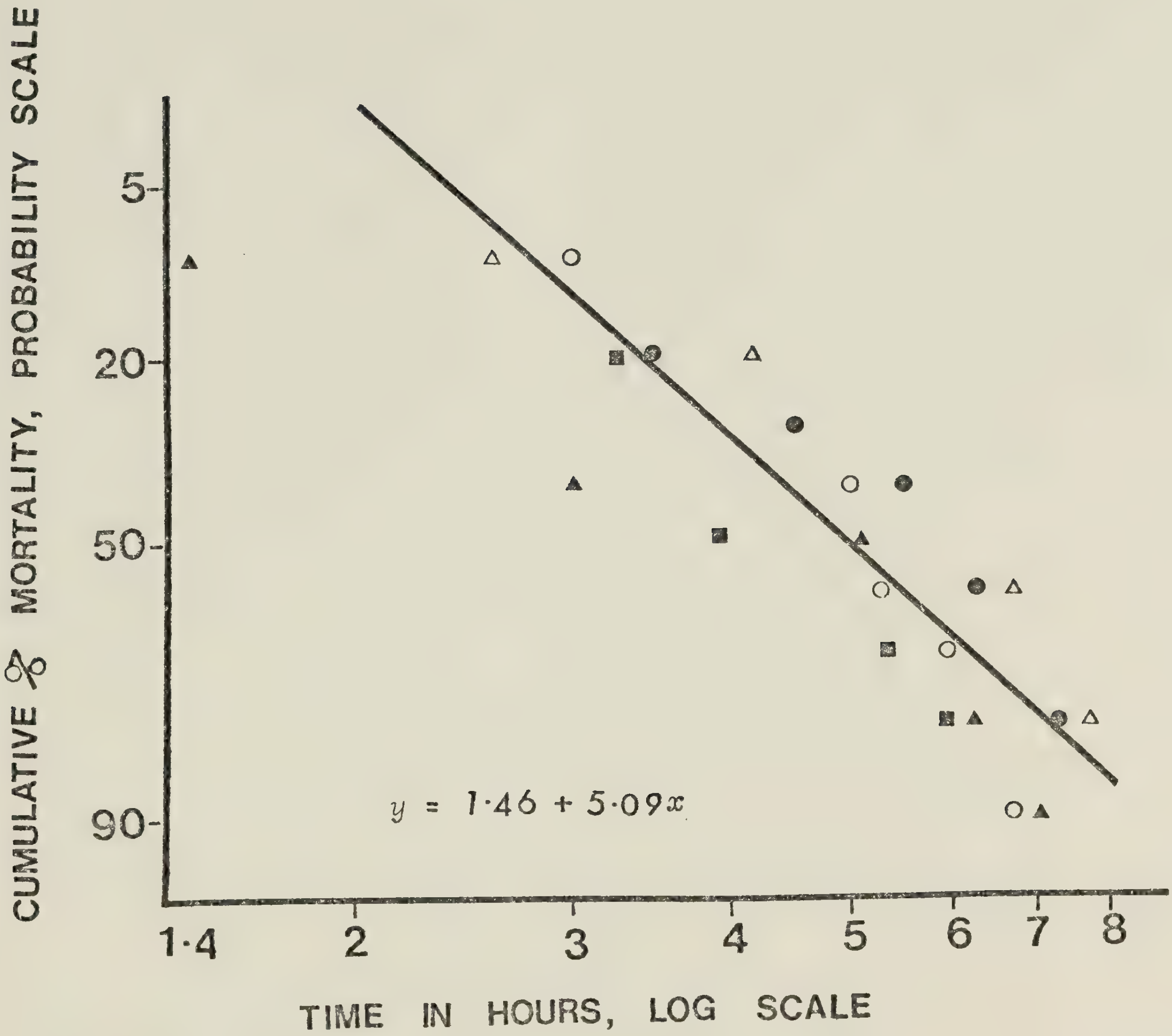

CUMULATIVE % MORTALITY, PROBABILITY SCALE
5
20
50
90
1·4
2
3
4
5
6
7
8
TIME IN HOURS, LOG SCALE
$y = 1{\cdot}46 + 5{\cdot}09x$

Figure 10. Probit regression line for estimating the LT50 (hours) of young guppies (C_2)[1] in 6 mg/l zinc.

Test Symbol	Source of fish
○	1st brood of ♀ 4
●	2nd brood of " 2
△	2nd brood of " 5
▲	3rd brood of " 4
■	4th brood of " 4

In the equation,

y = % mortality in probits

x = log of time (hours)

---

[1] Fish were postnatally exposed to 0.97 mg/l zinc for 12 hours before being tested.

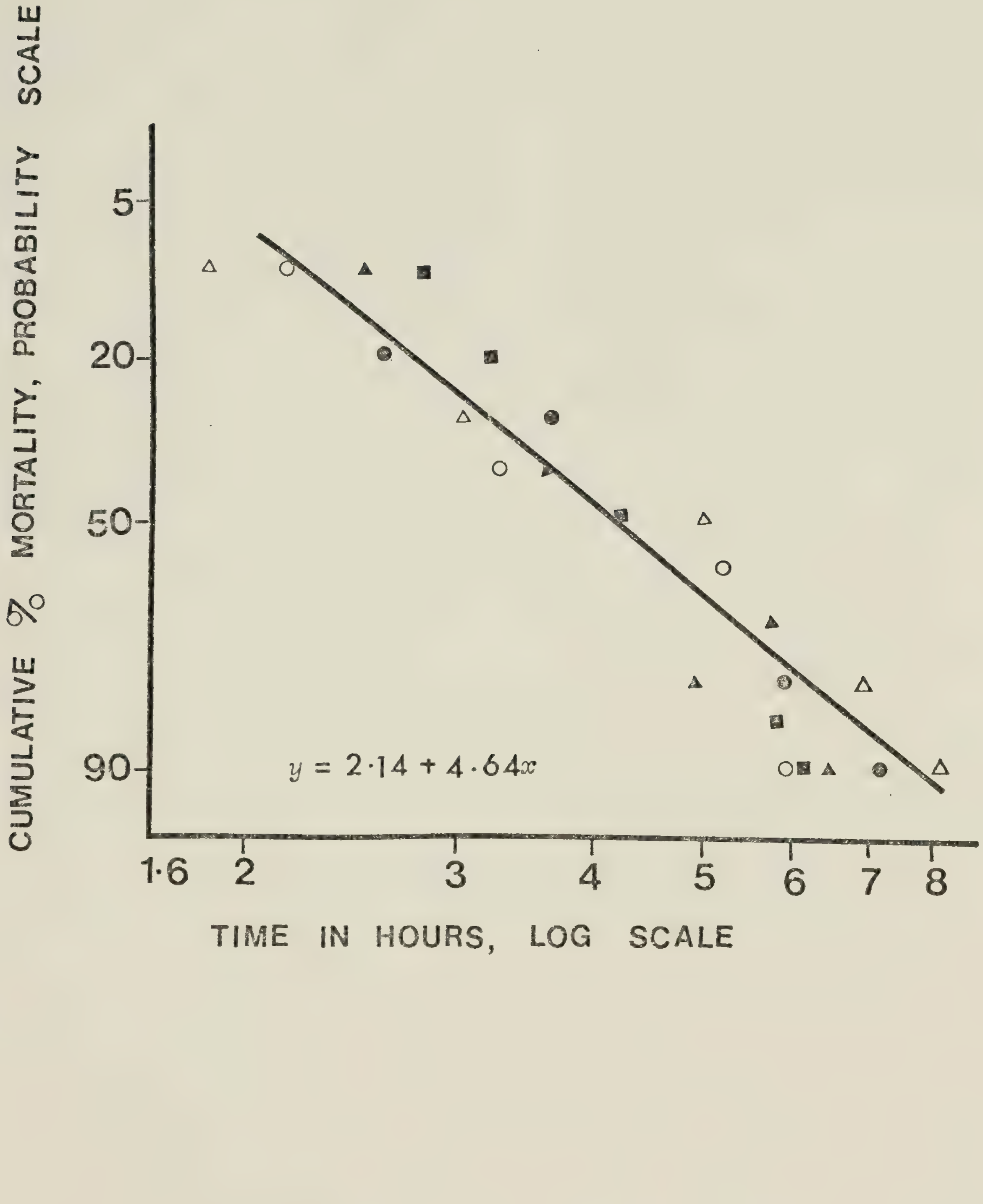

CUMULATIVE % MORTALITY, PROBABILITY SCALE
5
20
50
90
$y = 2{\cdot}14 + 4{\cdot}64x$
1·6
2
3
4
5
6
7
8
TIME IN HOURS, LOG SCALE

Figure 11. Probit regression line for estimating the LT50 (hours) of young guppies, that were prenatally exposed to 0.36 mg/l zinc, in 6 mg/l zinc.

Test Symbol	Source of fish
○	1st brood of ♀ 3
●	2nd brood of " 2
△	2nd brood of " 4
▲	3rd brood of " 4
■	4th brood of " 5

In the equation,

y = % mortality in probits

x = log of time (hours)

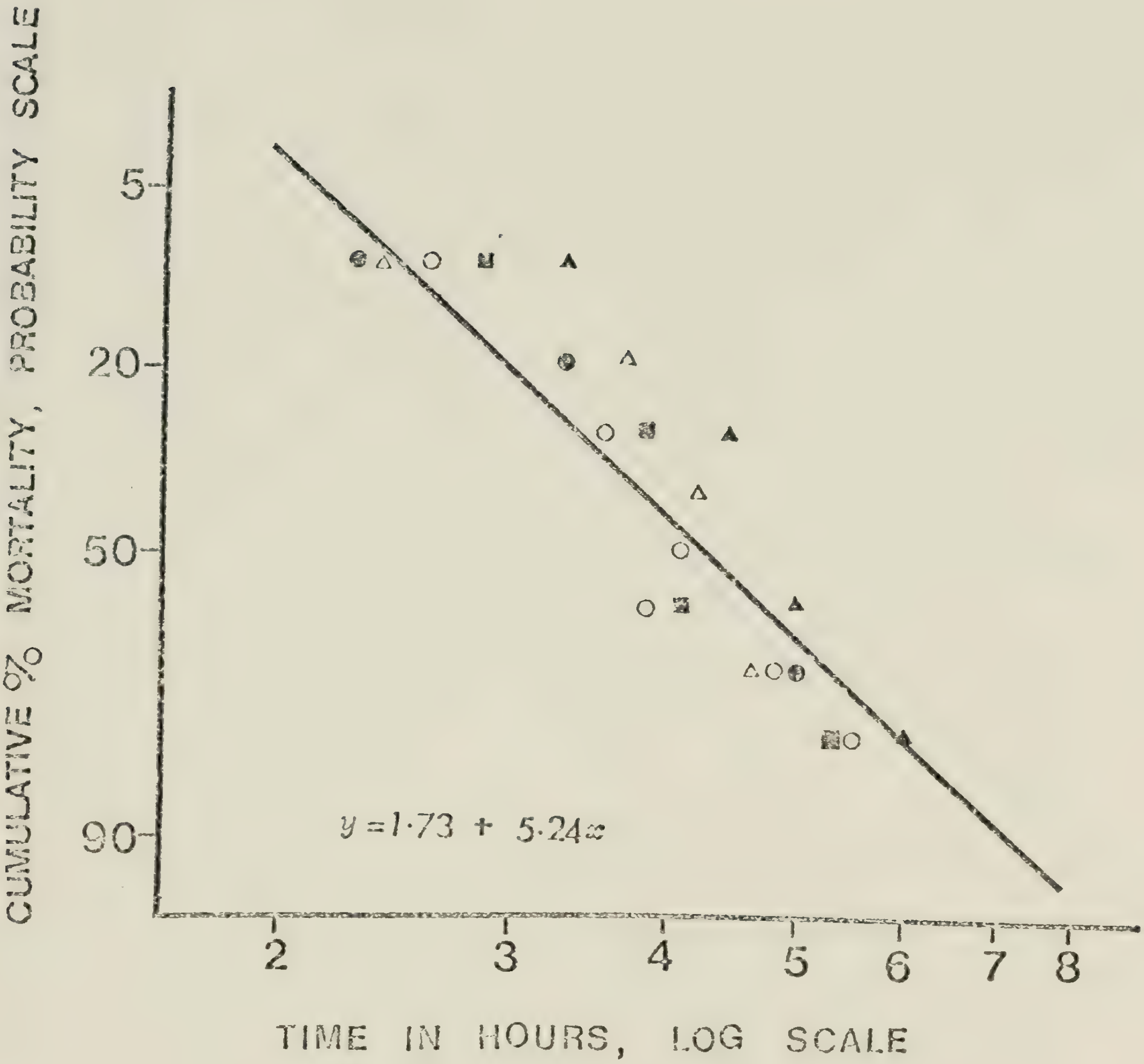

CUMULATIVE % MORTALITY, PROBABILITY SCALE
5
20
50
90
$y = 1{\cdot}73 + 5{\cdot}24x$
2
3
4
5
6
7
8
TIME IN HOURS, LOG SCALE

Figure 12. Probit regression line for estimating the LT50 (hours) of young guppies, that were prenatally exposed to 0.88 mg/l zinc, in 6 mg/l zinc.

Test Symbol	Source of fish
○	1st brood of ♀ 1
●	1st brood of " 4
△	1st brood of " 5
▲	2nd brood of " 2
■	[1] 3rd brood of " 2 4th brood of " 1

In the equation,

y = % mortality in probits

x = log of time (hours)

---

[1] Results combined.

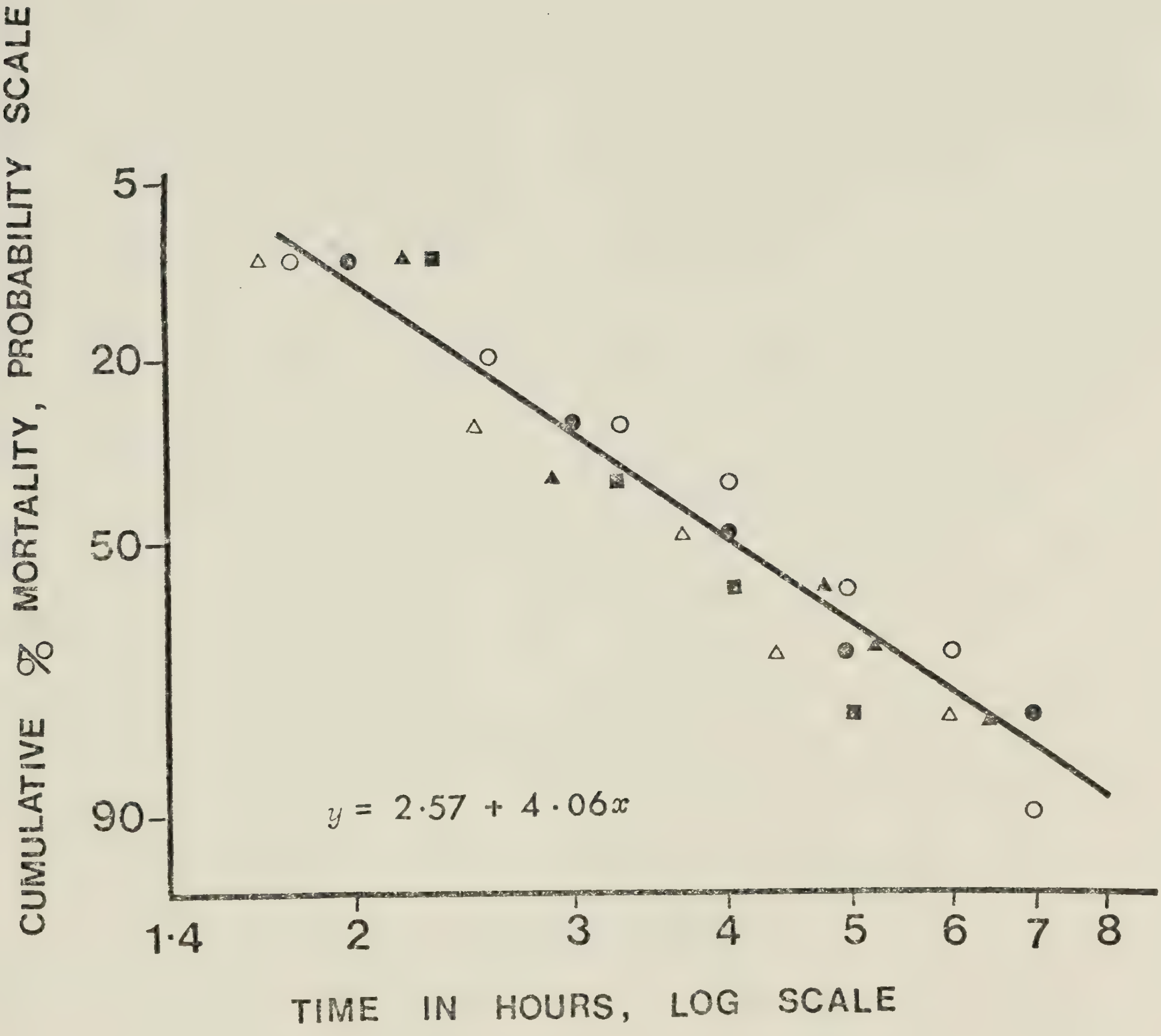

CUMULATIVE % MORTALITY, PROBABILITY SCALE
5
20
50
90
$y = 2{\cdot}57 + 4{\cdot}06x$
1·4
2
3
4
5
6
7
8
TIME IN HOURS, LOG SCALE

Figure 13. Probit regression line for estimating the LT50 (hours) of young guppies, that were prenatally exposed to 1.70 mg/l zinc, in 6 mg/l zinc.

Test Symbol	Source of fish
○	1st brood of ♀ 1
●	1st brood of " 2
△	1st brood of " 5
▲	[1] 3rd brood of " 2 [2]
	3rd brood of " 4
■	4th brood of " 3

In the equation,

y = % mortality in probits

x = log of time (hours)

[1] Results combined.

[2] Tested after recovery from anaesthesia.

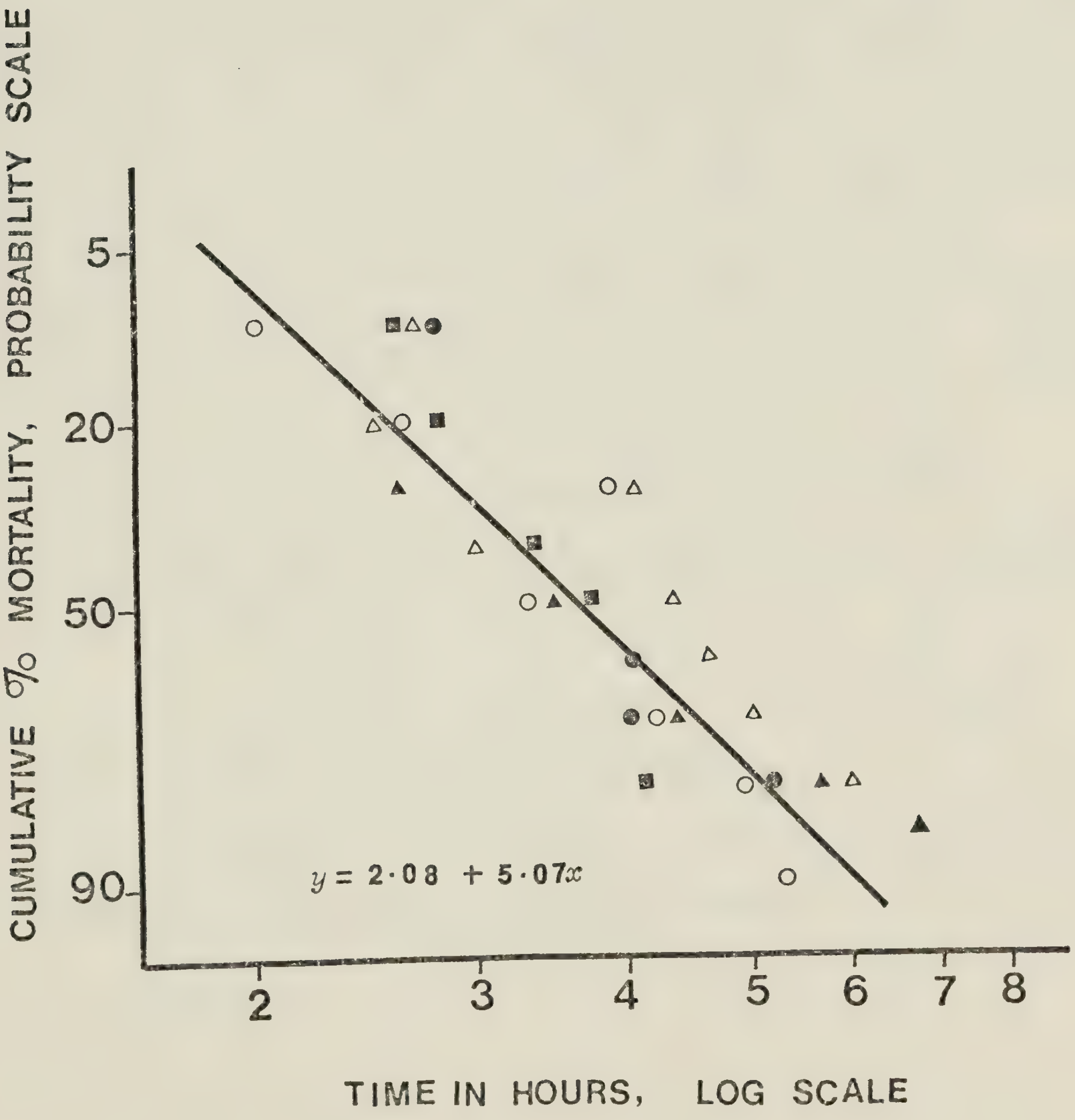

CUMULATIVE % MORTALITY, PROBABILITY SCALE
5
20
50
90
$y = 2 \cdot 08 + 5 \cdot 07x$
2
3
4
5
6
7
8
TIME IN HOURS, LOG SCALE

Table 2. Effect of prenatal or postnatal exposure to sublethal levels of zinc on the tolerance (as estimated by the LT50) of young guppies to an acutely toxic zinc concentration (6 mg/l).

Groups[1]:	C_1	C_2	0.36	0.88	1.70
LT50 (hours)[2]:	4.23	4.12	4.15	4.18	3.50
	4.10	4.05	4.45	5.25	4.10
	3.62	4.28	4.55	5.64	4.42
	4.15	4.90	4.00	3.84	3.55
	3.60	4.26	4.25	4.76	3.50
$\bar{X}$	3.94	4.32	4.28	4.86	3.81
LT50 (hours)[3]:	3.97	4.21	4.13	4.96	3.77

$$\begin{array}{ccccc} 1.70 & C_1 & 0.36 & C_2 & 0.88 \\ \cline{3-5} & & & & \\ \cline{1-4} \end{array}$$

Groups not underscored by the same line have significantly different LT50.

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[1] See Materials and Methods for details.

[2] LT50 estimated by Litchfield and Wilcoxon method.

[3] LT50 from log-probit regression lines.

than the other two parameters. It must be remembered however, that gonopodial swings were recorded only when the tip of the gonopodium was close to or touched the genital region of the female.

The general trend was for a lower courtship intensity in zinc solutions compared with controls but between group differences were not significant. It is therefore likely that the insemination frequencies may not have differed significantly from group to group. Within group differences were significant in test as well as control groups. In each case, the intensity of courtship was significantly higher during the first 3-week interval than in the remaining intervals and differences between the latter were not significant.

Results of the motor activity study (Figure 15) indicate that all fish groups exhibited more movement in the horizontal plane than in the vertical plane.

Motor activity decreased significantly with increase in zinc concentrations and between group comparison during each interval indicates that as early as the first interval, test fish were significantly less active than control fish. Within group comparisons indicate that test fish were significantly less active during the second, third and fourth intervals than in the first. Differences in activity of control fish from one interval to another were not significant.

Figure 14. Effect of chronic exposure to zinc on courtship behaviour of the male guppy.

Values are $\bar{X}$ ± 1SE (details in Appendix 8)

Concentrations are measured values

A = 0 mg/l zinc

B = 0.36 mg/l zinc

C = 0.88 mg/l zinc

D = 1.70 mg/l zinc

▒ Gonopodial swinging

□ Dorsal fin erection

⊟ Sigmoid reaction

Within group comparison of total courtship display (numbers refer to the intervals, order not necessarily increasing).

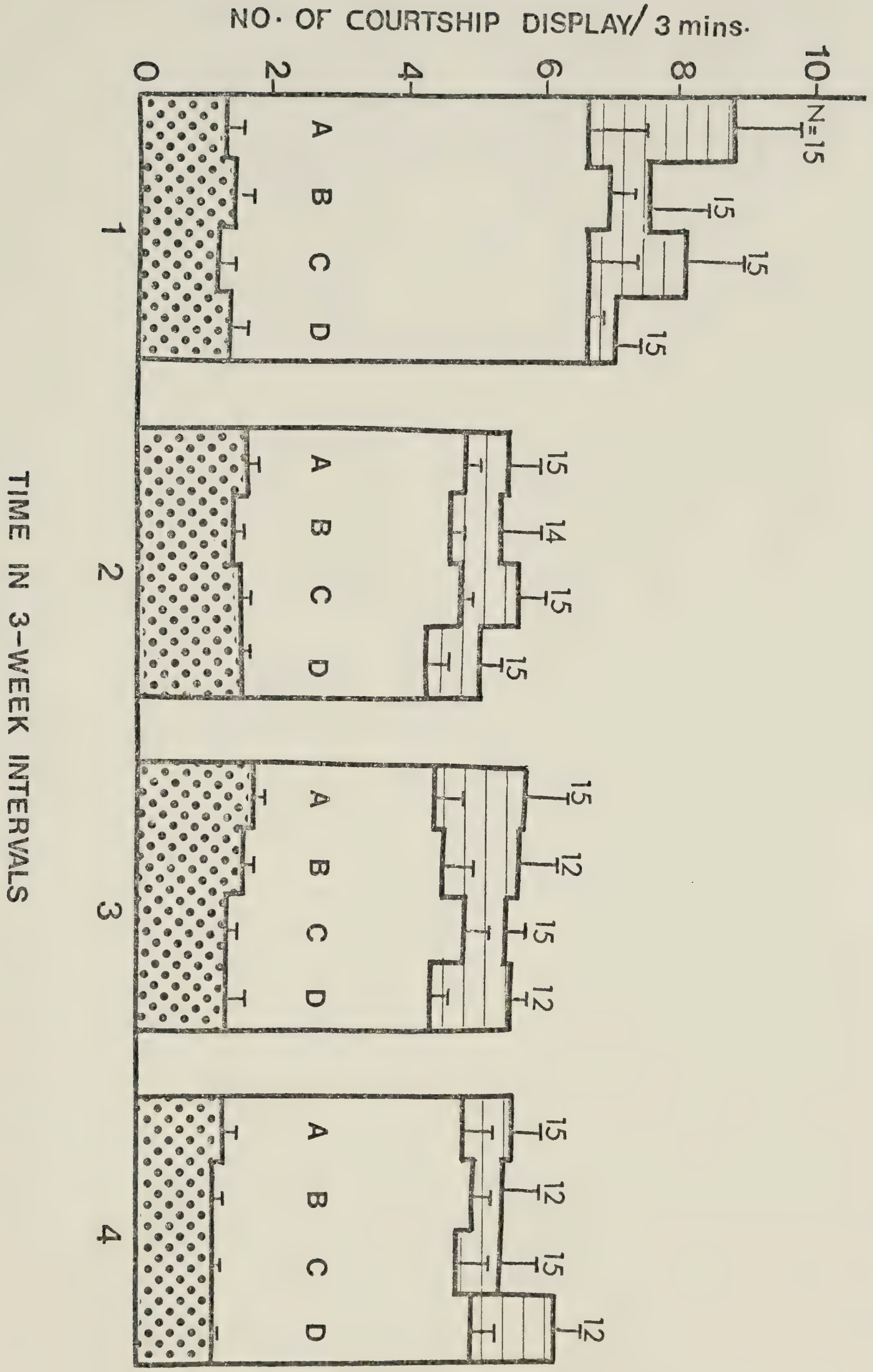
A: 1 $\underline{2 \quad 3 \quad 4}$

B: 1 $\underline{2 \quad 3 \quad 4}$

C: 1 $\underline{2 \quad 3 \quad 4}$

D: 1 $\underline{2 \quad 3 \quad 4}$

Intervals not underscored by the same line have significantly different means.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0
2
4
6
8
10
N=15
15
15
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15
14
15
15
15
12
15
12
15
12
15
12
A
B
C
D
1
2
3
4
TIME IN 3-WEEK INTERVALS

Figure 15. Motor activity of female guppies during chronic exposure to zinc.

Values are $\bar{X} \pm 1SE$ (details in appendices 9-11)

Concentrations are measured values.

A = 0 mg/l zinc
B = 0.36 mg/l zinc
C = 0.88 mg/l zinc
D = 1.70 mg/l zinc

□ Movement in the horizontal plane

░ Movement in the vertical plane

⊟ Sum of movement in both planes

Between group comparison (over the 12-week period)

□ : $D\quad C\quad B\quad A$
░ : $D\quad C\quad B\quad A$
⊟ : $D\quad C\quad B\quad A$

Between group comparison (during each 3-week period)

1st □ : $\underline{D\quad \rlap{\underline{}}C\quad B}\quad A$
░ : $\underline{B\quad C\quad D}\quad A$
⊟ : $\underline{D\quad C\quad B}\quad A$
2nd □ : $D\quad \underline{C\quad B}\quad A$
░ : $\underline{D\quad C\quad B}\quad A$
⊟ : $D\quad \underline{C\quad B}\quad A$
3rd □ : $\underline{D\quad \rlap{\underline{}}C}\quad B\quad A$
░ : $\underline{D\quad C}\quad \underline{B\quad A}$
⊟ : $\underline{D\quad C}\quad B\quad A$
4th □ : $D\quad C\quad B\quad A$
░ : $\underline{D\quad C}\quad B\quad A$
⊟ : $D\quad C\quad B\quad A$

Within group comparison of ⊟ (numbers refer to the intervals, order not necessarily increasing):

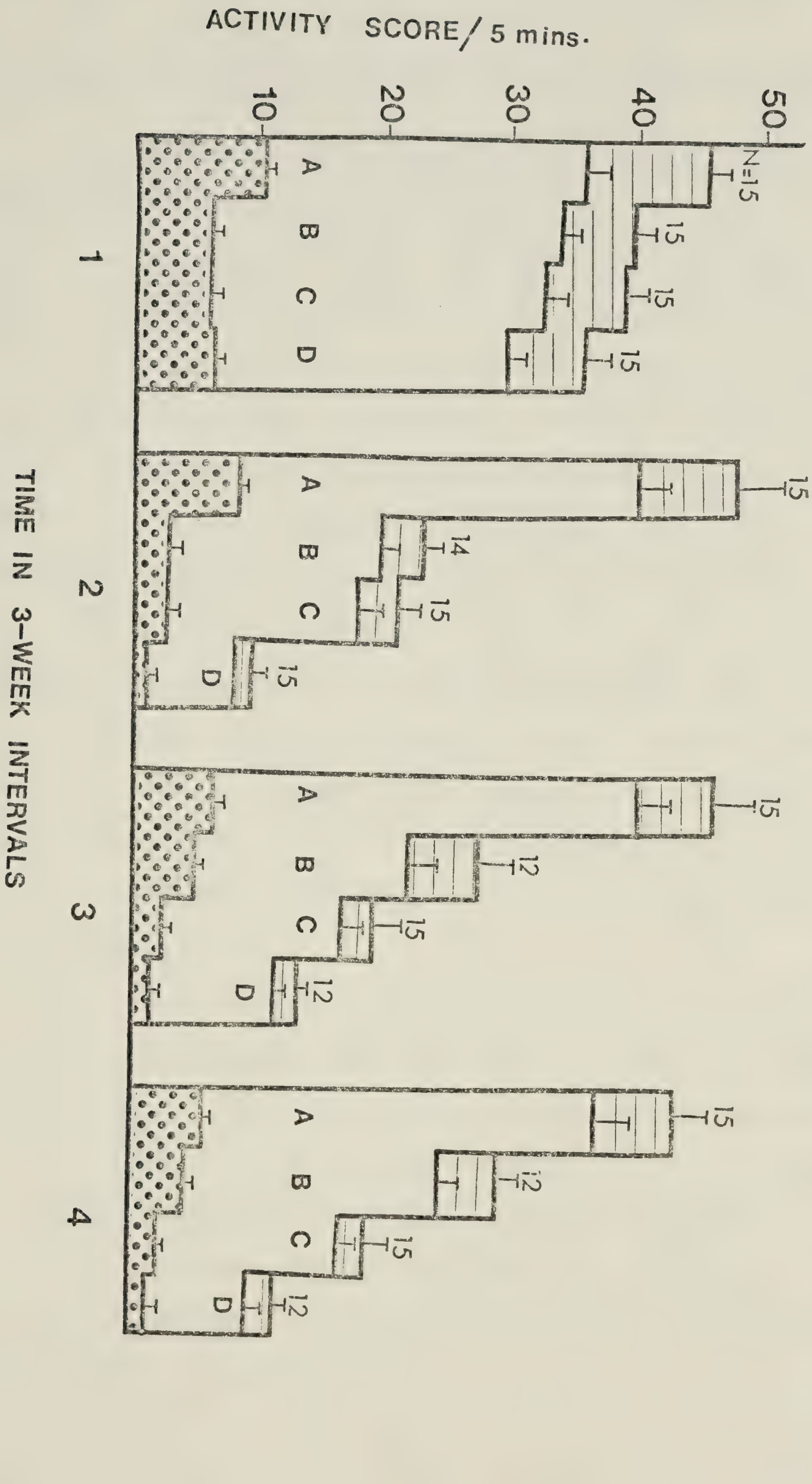
A: $\underline{1\quad 2\quad 3\quad 4}$

B: $1\quad \underline{2\quad 3\quad 4}$

C: $1\quad \underline{2\quad 3\quad 4}$

D: $1\quad \underline{2\quad 3\quad 4}$

Groups or intervals not underscored by the same line have significantly different means.


ACTIVITY SCORE/ 5 mins.
10
20
30
40
50
N=15
15
14
12
A
B
C
D
1
2
3
4
TIME IN 3-WEEK INTERVALS

DISCUSSION

Experiment 1. Acute toxicity of zinc to immature and adult fish

A typical plot of per cent mortality versus survival time shows an induction period during which no mortality occurs (Rubin and Elmaraghy, 1977), the length of this period decreasing with increase in zinc concentration (Chen and Selleck, 1969). The present investigation did not show a definite trend of decrease in induction period with increase in zinc concentration. However, the longest induction periods were recorded in the lowest zinc concentrations and fish that died at the higher concentrations had shorter survival times (Figures 1 and 2).

When a fish is exposed to zinc, the gills are thought to accumulate zinc down a concentration gradient (Hodson, 1974). Fish in higher zinc concentrations could therefore take up more zinc and as a result, gill damage and the consequent development of tissue hypoxia would occur faster. This could explain the shortening of survival times at the higher zinc concentrations.

This study estimated the 10-day LC50 of adult and immature guppies to be between 4.57 and 5.01; 2.40 and 2.88 mg/l zinc respectively. Although a lot of work has been done on the acute toxicity of zinc to fish, differences in a) chemical and physical properties of test water, b) duration of tests, and c) test species, limit the number of comparisons which can be made between tests and often detract from the significance of such comparisons.

Pickering and Henderson (1966) reported the 96-hour LC50 of adult guppies to be 1.27 mg/l zinc. The pH and temperature of their test, 7.5 and 25°C respectively, were similar to those (7.4 and 25°C) of this study. The total hardness of their test water was only 20 mg/l. Therefore, the 10-day LC50 (4.57-5.01 mg/l zinc) estimated for adult guppies in this study, at a total hardness of 95 mg/l follows the expected trend of increase in tolerance with increase in hardness.

Mount (1966) estimated the 96-hour LC50 of the fathead minnow to be 12.5 mg/l zinc. His test conditions of pH, 7; temperature, 25°C; total hardness, 100 mg/l, were similar to those of this study. It is therefore probable that fatheads are more tolerant of acute zinc toxicity than guppies. However, Pickering and Henderson (1966) measured the tolerance of these two species under the same test conditions (soft water) and they reported the 96-hour LC50 for guppies and fatheads as 1.27 and 0.87 mg/l zinc respectively. The difference in tolerance was non-significant. This finding can still be reconciled with the conclusion that was reached by comparing the value reported for the fathead minnow by Mount and that estimated for the guppy in the present investigation. There is no doubt that the longer exposure period of 10 days of the present investigation contributed to the lower LC50 value obtained for the guppy since fish continued to die after the 96-hour mark. This value may in fact be close to that level at which half of the fish may be expected to live indefinitely (ie. the Incipient lethal level, Sprague, 1969). Also, a

change in one of the properties of the test water may not necessarily alter the tolerance (to zinc) of different species to the same extent. Consequently, a significant difference in the tolerance of two species in soft water could become non-significant in hard water if the increase in hardness produces a greater increase in the tolerance of the less tolerant species. For example, an increase in hardness from 20 to 360 mg/l produces a 38.4 X and a 7.4 X increase in tolerance of fatheads and bluegills respectively (Pickering and Henderson, 1966). As a consequence, while bluegills are significantly more tolerant than fatheads in soft water, the difference is much smaller in hard water (Pickering and Henderson, 1966) and may become non-significant. It is probable that a decrease in hardness from 100 to 20 mg/l produces a larger decrease in the tolerance of fatheads compared with guppies, so that in soft water the large difference in tolerance disappears. It is also interesting to note that while a temperature decrease from 25°C to 15°C significantly increases the 96-hour LC50 of fatheads in soft water from 0.77 to 2.55 mg/l zinc, the increase in the bluegill was only from 5.46 to 6.44 mg/l zinc (Pickering and Henderson, 1966).

Results of the 10-day LC50 (table 1) show adults to be more tolerant than immature guppies. Variation in tolerance among different stages of the life cycle has been reported in the literature. Eggs of the fathead minnow are more tolerant than one-day-old fry (Pickering and Vigor, 1965). In the

zebrafish (*Brachydanio rerio*), Skidmore (1965) found that newly laid eggs and newly hatched fish were the most and least tolerant stages respectively. Skidmore (1966) demonstrated that the higher tolerance of eggs of the zebrafish was not due to protection by the outer egg membrane.

Attempts have been made to relate differences in tolerance to zinc of different life cycle stage of the zebrafish to differences in weight-specific oxygen uptake (Skidmore, 1967). Perhaps a study that would be more directly related to the problem is one that examines the 'performance' of these different stages under conditions of deficient oxygen. The consensus of opinion among researchers is that acute zinc toxicity in fish is due to the development of internal hypoxia. Differences in the capability to compensate for or to adapt to the hypoxic conditions might be the key factor in the tolerance differences observed for different stages of the life cycle.

Although the mode of action of zinc (during acute toxicity) is essentially external, Chapman (1973) speculates that there is the need for the zinc ions to make close contact with, or even enter the epithelial cells of the gills. Since gill area per gram of fish decreases with increasing fish size (Muir, 1969), smaller fish may therefore be expected to present a relatively larger surface area on which the toxicant can act and therefore increase the extent of the development of internal hypoxia. If intraspecific differences are large enough, this factor could contribute to tolerance differences. Because

immature fish are smaller and are growing to attain adult size, their oxygen requirements per gram body weight might be greater than those of adult fish and as a consequence, immature fish could be more adversely affected.

Whereas this study found a significant increase in zinc tolerance with a 16-fold increase in size, Cairns and Scheier (1959) found no significant difference in tolerance of bluegills with a 57-fold increase in size. Their results actually showed a slight decrease in tolerance with increase in size. The difference in findings of both studies might be simply due to species differences.

Zitko and Carson (1977) performed monthly acute toxicity studies on juvenile Atlantic salmon from September to July and suggested that the higher tolerance of these fish during the winter might be due to their lower metabolic rate. They also speculated that the increased sensitivity in the spring might be due to 'increased metabolic rate and physiological changes during the initial stages of the parr-smolt transformation'.

If polluting levels are within the range of tolerance of the guppy population, a significant tolerance difference, as found in the present study, would suggest that immature fish could be more adversely affected. The effect could be one of a change in the age-structure of the population in addition to the reduction of population size. One would not expect this change to last long when normal or near-normal conditions return since the surviving population will contain many reproducing individuals.

Experiment 2. Effect of zinc on reproduction

The present investigation reports that chronic exposure to 0.88 and 1.70 mg/l zinc, concentrations which approximately represent 0.2 and 0.4 of the 10-day LC50 respectively, caused a significant decrease in brood size. The brood sizes recorded for test fish are not unusual and can occur under normal circumstances (Rosenthal and Rosenthal, 1950; Rosenthal, 1952). Brood size of guppies shows a cyclic variation: initially the value is low; increases to a maximum and then decreases to a minimum. The unusual feature was that most of the exposed females consistently produced smaller brood sizes (Appendix 6).

Brungs (1969) observed a significant decrease in fecundity of the fathead minnow during a 10-month exposure to 0.18, 0.34, 0.68 and 1.3 mg/l zinc. Although the two highest concentrations appear comparable to those reported in the present investigation, the former were only 0.07 and 0.14 respectively, of the 96-hour LC50 determined by Brungs. At 2.8 mg/l zinc, a concentration that was 0.30 of the 96-hour LC50, egg production was completely inhibited in the fathead minnow (Brungs, 1969). No valid conclusions can be drawn, with respect to differences in tolerance of chronic zinc toxicity of the two species, because of differences in the duration of the acute and chronic studies. One might, in fact, expect the effect of zinc to be greater in the guppy because of the viviparous mode of reproduction. However, the apparently higher tolerance of guppies may be real and could be entirely due to species tolerance differences. Speranza *et al.* (1977) found a significant decrease in the

number of eggs produced by the zebrafish following a 9-day exposure (a period in which gametes were maturing) to 5 mg/l zinc. They also found that the effect was reversible on return to a zinc-free environment. In the present investigation, involving the guppy, a decrease in the reproductive capacity did not occur until the second parturition period. This time interval probably represents the time required for the metal to accumulate to levels high enough to exert a noticeable effect. Whether or not the effect is also reversible in the guppy is not known at present.

A direct relationship between fecundity and the amount of food consumed has been demonstrated in the rainbow trout (Scott, 1962; Bagenal, 1969), and in the guppy (Hester, 1963). Although the present investigation did not quantify food consumption, information from the literature seem to indicate that exposure to sublethal levels of zinc could reduce the amount of food consumed. Cairns and Loos (1967) reported a decreased feeding rate in the zebrafish during a 96-hour exposure period. If the effect is long lasting, exposure to sublethal levels of zinc even under conditions of abundant food supply could result in a decrease in the overall amount of food consumed. Crandall and Goodnight (1962) observed that some guppies did not feed normally after 30 days exposure to 1.15 mg/l zinc. Minnows (*Phoxinus phoxinus*) exposed to 0.05-0.30 mg/l zinc consumed less food than controls (Bengtsson, 1974a). Watson and McKeown (1976a) observed that although rainbow trout exposed to 1.12 mg/l zinc consumed as much food as controls,

growth rate in the former was significantly lower. It would appear, therefore, that the problem of reduced fecundity (and reduced growth rate) is due to reduced energy availability and consequently, reduced food assimilation and energy transformation (Watson and McKeown, 1976a; Bengtsson, 1974a) would produce similar results as reduced food consumption.

The mechanism by which zinc suppresses food consumption is not known. Application of 16 mg/l $ZnSO_4$ to the palatal organ of the carp, *Cyprinus carpio*, depressed the response of salt and sugar receptors (Hidaka, 1970). This concentration (=6.5 mg/l zinc) is high compared with those in the present investigation. However, in Hidaka's study the zinc salt was applied for only ten seconds. Lower concentrations such as those used in the present investigation could probably exert a similar effect with increase in exposure time. Zinc-induced degenerative changes in the palatal organ, if they occur, might also produce a similar effect. Such responses could probably account, partially at least, for the reduced food consumption during chronic exposure of fish to zinc. No information is available on the effect of zinc on the efficiency of assimilation.

Hiltibran (1971) demonstrated a phosphate 'uncoupling' effect for low levels of zinc in the mitochondria of bluegill liver. He speculated that the observed effect might be due to 'some alteration in the sequence of the incorporation of inorganic phosphate into tissue organic phosphate . . . '. The consequence could be impaired energy transformation.

Mention has already been made of degenerative changes in the guppy resulting from chronic exposure to zinc (Crandall and Goodnight, 1963). Similar exposures have also caused degenerative changes in the spinal cord of the minnow, *Phoxinus phoxinus* (Bengtsson, 1974b). The question of additional tissue repair therefore arises in exposed fish. Dilation of renal tubules in chronically exposed guppies (Crandall and Goodnight, 1963) is probably indicative of increased excretory demands. Adjustment to the presence of the toxicant could call for the establishment of some kind of detoxicating mechanism; which in its simplest form could involve chelation by proteins or storage of accumulated zinc in inert tissues. The comparatively larger amounts of accumulated zinc in such tissues as the gill arch and the opercular bone of the bluegill (Mount, 1964) and the spinal column of chinook salmon fingerlings, *Oncorhynchus tshawytscha*, (Joyner and Eisler, 1961) are probably the result of a redistributing mechanism. Some of these processes may be energy-consuming. If energy transformation is impaired while the energy cost of metabolism and tissue repair rises, it becomes obvious that exposed fish can only budget comparatively smaller amount of energy for such non-maintenance processes as growth and reproduction.

Speranza et al. (1977) reported a significant decrease in the number of viable eggs produced by zebrafish that were exposed to zinc. This effect was probably due to damage to the gametes prior to fertilization, since spawning occurred in a zinc-free environment. Damage to gonadal products, apart

from reducing fertilization success or egg viability, could prevent successful completion of later developmental stages. Results obtained in the present investigation indicate very strongly that embryonic development is another area where the toxicant can exert an effect. There were three cases in which a mass of tissue, representing an undifferentiated or a dedifferentiated embryo (as noted by Rosenthal and Alderdice, 1976), was found. Some young were born dead, smaller than usual, had not uncurled from the embryonic posture and the yolk had not been completely absorbed. Interestingly, there was no instance where the brood contained only these individuals. Since superfoetation (ie. a second brood is not fertilized until the first is delivered) does not occur in the guppy (Turner, 1937), it is probable that the toxicant had the effect of either slowing down, arresting or even reverting (dedifferentiation) development. The level of toxicant at which this occurs will vary from female to female and from embryo to embryo.

Damage to gametes prior to fertilization may not solely account for the observed effects. Arrested development could result 'if the available energy budget is sufficient only for maintenance of cells in their current state of organization' (Rosenthal and Alderdice, 1976). It is therefore likely that the developing young also faced some kind of an 'energy crisis'. Intensive growth and differentiation of embryos of the guppy take place during the second and third weeks of the gestation period and during this time, maximal nourishment of embryos is

necessary (Stolk, 1951). Nutrient required by the developing embryos for maintenance metabolism comes from the mother (Scrimshaw, 1945). If the assimilation efficiency and/or energy transformation of the mother is impaired, the amount of nutrients made available to the young from her and the number of young she can therefore successfully support could be reduced.

Eggs, like other tissues, may be capable of accumulating zinc. If the levels are high, they could exert the same 'uncoupling' effect that has been observed in the adult fish. Because of the intimate vascular connection in the guppy between embryo and mother (Purser, 1938; Scrimshaw, 1945), substances in the blood of the mother, including zinc, could eventually reach the embryo. Placental transfer of zinc from mother to embryo has been demonstrated in the rat (Feaster *et al.*, 1955). That dead fry had not completely absorbed the yolk indicates that the problem might be one of food utilization or energy transformation and not due to reduced food availability.

Decreased energy availability resulting from one or more of the following: decreased amount of nutrient made available to young from exposed mother, impaired energy transformation of young induced by zinc or increased cost of tissue maintenance could account for the decreased size of young (at birth) produced by mothers exposed to 0.88 and 1.70 mg/l zinc.

Another point in the reproductive cycle of fish where zinc has been shown to have an adverse effect is the success of hatching. Brungs (1969) observed that the hatching process

began prematurely in fathead minnow eggs exposed to 2.8 mg/l zinc and before hatching was complete, most of the fry had died. Rosenthal and Alderdice (1976) suggested that unsuccessful hatching as a result of exposure to toxicants might be due to reduced embryonic movement within the egg and as a result, the hatching enzyme becomes concentrated in the head region. Although this local concentration of the hatching enzyme could explain the premature hatching observed by Brungs, it is doubtful if this mechanism will play a significant role under the conditions of his tests since the eggs were in the zinc-polluted environment for about three days only before hatching began.

Within the range of concentrations studied, the toxicant did not affect the intervals between broods. This means that the frequency of 'spawning' did not contribute to the zinc-induced reduction of the total number of young produced by exposed fish. Events that occur during intervals between broods include 1) development of middle-sized oocytes into large oocytes, 2) fertilization, 3) development and growth of embryos and, finally, 4) parturition. A 'no-effect' on the intervals between broods could be understood to mean that none of the mentioned events was slowed down significantly. Results obtained in this study, however, indicate that the development of some young was slowed down. Intervals between broods may not, therefore, be a sensitive indicator of the effect of zinc on the events mentioned above. Guppies, under normal circumstances, show a lot of variability in the intervals between

broods. Intervals ranging from 28 to 60, 22 to 44 days (Turner, 1937) and 27 to 59 days (Yamagishi, 1976) have been reported for the same female guppies respectively. A variability of that magnitude could overshadow any effects the toxicant may have on the events mentioned.

Brungs (1969) reported a zinc-induced reduction in the frequency of spawning in the fathead minnow. The number of spawnings per female during a 10-month exposure period was 0.5, 0.5 and 11 in 1.3, 0.66 mg/l zinc and controls respectively. Speranza *et al.* (1977) found that after a 9-day exposure to 5 mg/l zinc, zebrafish showed a delay in spawning. The latter investigators suggested that the observed effect might be due to a delay in gonadal maturation. It is not known if intervals between broods in the guppy would have increased significantly if exposure was continued for longer periods.

Zinc might also inhibit the spawning process per se. This effect has been reported for bluegills that were exposed to 0.24 mg/l zinc (Sparks *et al.*, 1972). The significance of this finding is that even if the number of eggs produced per spawn is unaffected, changes in the frequency of spawning (or parturition) could, over time, reduce the reproductive success of fish populations.

Experiment 3. Effect of previous exposure to zinc on the tolerance of young fish

Results of the acclimation studies (table 2) seem to indicate that there apparently is an optimal level of zinc at which prenatal exposure increases the tolerance of young

fish to acute zinc toxicity.

The second control group (C_2), in which young were postnatally exposed to 0.97 mg/l zinc for 12 hours before being tested, survived slightly longer than the C_1 group. Although the difference was not significant, it apparently followed the trend shown in other acclimation studies (Pickering and Vigor, 1965; Sinley *et al.*, 1974; Lloyd, 1960).

This study is certainly inconclusive and the finding should not be overemphasized. The young fish were not all from the same parturition periods and therefore some had come from females that were exposed longer than others. Some young were tested after recovery from anaesthesia because of the small number of young produced by exposed fish. Increased survival time may not necessarily mean increased tolerance (Hodson, 1974) and a more valuable study would be one that involves LC50 determinations so that the response of each of the various groups is measured over a range of toxicant concentrations. In order to obtain enough young to carry out LC50 determinations, one may need to expose the adults to zinc concentrations that are lower than those used in the present investigation, in addition to using a much larger number of adult fish.

The mechanism of acclimation of fish to zinc and other heavy metals is yet to be understood and there hardly is any speculation in the literature. If zinc has to penetrate the epithelial cells of the gills before it can exert a toxic effect, then one can suggest that the increased tolerance of previously

exposed fish may be due to a decrease in zinc uptake as a result of the previous exposure. Another probable explanation is that previous exposure to zinc lowers the oxygen demand of the fish so that the amount of zinc that reaches the gill epithelium is reduced.

Selective mortality is one of the factors by which a population may increase its tolerance (Skidmore, 1964). If prenatally exposed guppies are more tolerant of acute zinc toxicity, then selective mortality may be a probable mechanism. It is easy to imagine that during the gestation period, weak embryos are eliminated and the young that survive are more hardy and probably more tolerant of adverse conditions, including an acutely toxic zinc concentration.

Experiment 4. Effect of chronic exposure to zinc on courtship behaviour and motor activity

a) Courtship behaviour

Courtship intensity did not differ significantly between control and test groups. Although mating was observed on a few occasions only, the conclusion could be made that the frequency of insemination was the same in all groups since 'there may very well be a high correlation between courtship behaviour and the ability and opportunity for copulation' (Clark and Aronson, 1951). The results suggest therefore that the small brood sizes of test groups were not due to a decreased frequency of insemination.

The courtship figures reported in this study are high compared with those of Warren (1973). Individual differences,

which may increase when interpopulation comparisons are made, could account for the differences in both studies. Test tanks in the present investigation held one male and one female fish only. Consequently neither fish would spend time on aggressive behaviour towards other fish. Increase in aggression and decrease in courtship behaviour resulting from increase in density of a population of guppies (Warren, 1973) might understandably represent a response to maintain the population density at an optimal level. However, physiological responses, such as a decrease in ovarian stages, might play a more efficient role in this respect; in a fish where one insemination could fertilize several successive broods (Purser, 1938; Winge, 1937). It is therefore probable that courtship activity in Warren's study merely decreased as a result of increased aggression. If this is so, then in the present investigation one would expect the opposite since aggression within the pair would be expected to be low; because of the tendency for guppies to attack their own sex more frequently than the other sex (Warren, 1973).

Vision plays an important role in courtship activity of the guppy (Breder and Coates, 1935). In the present investigation, where the volume of the medium was only 4.5 litres, it would be expected that the female might not escape the notice of or escape easily from the persistently courting male.

One feature of the courtship display in the present investigation, which can not be fully accounted for at this time,

is the marked reduction in both control and test groups after the first 3-week interval. The result gives one the impression that males tend to court 'inexperienced' females more vigorously. A probable explanation is that because of their history of isolation from males, virgin females may need time to adapt to the presence of the males before responding to their courtship displays. This would mean that virgin females are less receptive and might therefore be courted for longer periods. Liley's study (1966) indicates that this might not be the case: the female's response appears to affect the quality and quantity of the male courtship. More receptive females would therefore be expected to be courted more vigorously than non-receptive ones. Virgin females may, in fact, be more persistently receptive than non-virgin females and the receptivity of the former group drops as embryonic development begins (Liley, 1966). The duration of this period of higher receptivity (before embryonic development begins) was estimated by Liley as 18 days. This period forms a major fraction of the first 3-week interval and could account for the significantly higher figures recorded during this period.

b) Motor activity

The reduced activity of guppies during chronic exposure to zinc, observed in this study, might be the result of decreased energy availability. Mention has already been made of the various factors that could reduce energy availability in exposed fish. It is also probable that the observed effect

is only a result of altered behaviour. There was the tendency for exposed fish to move only within the lower half of the test tanks and sometimes, they simply rested at the bottom. When they did move, however, they did not appear to have any difficulty in swimming or maintaining equilibrium. Crandall and Goodnight (1962) had earlier observed that some of the guppies exposed to 1.15 mg/l zinc, in addition to showing stunted growth, were comparatively inactive and appeared to have difficulty in swimming and maintaining equilibrium. Minnows, *Phoxinus phoxinus*, exposed to 0.20 mg/l zinc or more, were not only less active but also showed a tendency to swim near the bottom and form dense shoals (Bengtsson, 1974b). The above investigator also observed that the exposed fish were highly excitable.

Sublethal levels of zinc (0.20 mg/l and above) have been shown to cause degenerative changes in the vertebrae and spinal cord of the minnow (Bengtsson, 1974b). Such an effect could partially account for the depression of activity in exposed guppies as observed in this study and in Crandall and Goodnight's study. The effect would be expected to be more pronounced in the latter study since the guppies were exposed when they were only a day or two old.

Since exposed fish in the present investigation did not seem to have difficulty in moving, one can not speculate on their ability to escape from predators. The other studies seem to indicate that exposed fish may be less successful at escaping predation. If exposed fish form dense shoals, as Bengtsson has noticed, coupled with a probable reduction in ability to

swim, exposed populations may become more easily noticed and preyed upon.

Apart from a reduction in activity, observations in the present investigation and from Bengtsson's study seem to indicate that chronic exposure to zinc could alter movement patterns of exposed fish to the extent that only part of the habitat is fully utilized. A spatial restriction could artificially cause an increase in population density and reduced food availability. Behavioural and/or physiological changes could occur as in usually observed in over-crowded populations. These changes, even in the absence of predation, could cause numerical changes in the population.

Although courtship behaviour involves movement, a zinc-induced decrease in motor activity did not produce a decrease in courtship activity. Motor activity of males was not measured but it is unlikely that females would have been differentially affected; in which case exposed males may not have found it more difficult courting exposed females (when compared with unexposed males courting unexposed females).

SUMMARY

Results of the present investigation indicate that in the guppy,

1) immature fish are less tolerant of acute zinc toxicity than adult fish.
2) chronic exposure to zinc a) decreases the number of young produced per female, b) does not affect the intervals between broods, c) decreases the size of young at birth.
3) there apparently is an optimal level of zinc at which prenatal exposure increases the survival time of young in an acutely toxic zinc concentration.
4) chronic exposure to zinc a) does not affect the intensity of courtship behaviour, b) decreases motor activity and exposed fish tend to stay near the bottom of the tank.

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APPENDIX I

Physical and Chemical Properties of Water in Experiment I.

Fish	Characteristic (unit)	Triplicate LC50[1]	Mean	N	Range
Immature	pH	A	7.5	92	7.2- 8.0
		B	7.3	143	6.8- 7.7
		C	7.3	121	6.9- 7.7
	Dissolved oxygen (mg/l)	A	6.8	70	5.9- 7.3
		B	7.1	26	6.8- 7.4
		C	6.9	138	5.8- 8.8
	Total hardness (as $CaCO_3$, mg/l)	A	97.7	10	84 -112
		B	96.5	10	88 -100
		C	90.9	10	80 -120
	Total alkalinity (as $CaCO_3$, mg/l)	A	19.3	8	17 - 20.8
		B	18.0	8	16 - 23.1
		C	17.8	8	17 - 20.5
	Temperature ($^{\circ}C$)	A	25.6	126	25.1- 26.4
		B	25.7	142	25.2- 26.1
		C	25.7	143	25.3- 26.1
Adult	pH	A	7.3	132	7.0- 7.8
		B	7.4	132	7.0- 8.0
		C	7.4	130	7.0- 7.8
	Dissolved oxygen	A	6.8	60	5.8- 7.8
		B	7.0	26	6.5- 7.4
		C	7.0	26	6.6- 7.8
	Total hardness	A	98.3	10	85 -118
		B	96.4	10	84 -112
		C	91.8	10	86 -104
	Total alkalinity	A	20.3	8	18.6- 24
		B	17.8	8	16.8- 20
		C	16.5	8	16.0- 19
	Temperature	A	25.6	140	25.4- 26
		B	25.7	149	25.3- 26
		C	25.8	143	25.2- 26.2

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[1] A, B and C represent the three sets of LC50 determinations. The mean values represent measurements for 6 experimental and 1 control tanks run in duplicates.

Appendix 2

Zinc Concentrations (mg/l) and Mortality Data in Experiment 1.

Fish	LC50 determination	Nominal zinc conc.	Measured zinc conc. in duplicate tests		% Mortality in tests		$\bar{X}$ of tests 1 and 2	
			1	2	1	2	Measured zinc	% Mortality
Immature	A	1.23	1.08	1.06	10	0	1.07±0.08	5
	B		1.07	1.14	10	10	1.11±0.12	10
	C		1.12	1.11	10	0	1.12±0.14	5
	A	1.73	1.60	1.60	10	30	1.60±0.07	20
	B		1.63	1.67	20	20	1.65±0.12	20
	C		1.61	1.51	0	20	1.56±0.16	10
	A	2.63	2.56	2.63	40	50	2.60±0.18	45
	B		2.68	2.54	40	60	2.61±0.18	50
	C		2.40	2.51	40	60	2.46±0.19	50
	A	3.73	3.45	3.52	60	70	3.49±0.18	65
	B		3.68	3.72	50	70	3.70±0.13	60
	C		3.55	3.53	70	60	3.54±0.14	65
	A	4.23	3.94	3.87	90	70	3.91±0.22	80
	B		4.27	4.25	90	80	4.26±0.17	85
	C		4.03	3.99	70	90	4.01±0.18	80
	A	4.73	4.32	4.32	100	80	4.32±0.15	90
	B		4.66	4.68	80	90	4.67±0.27	85
	C		4.35	4.37	100	80	4.36±0.22	90
Adult	A	2.73	2.72	2.76	0	10	2.74±0.16	5
	B		2.76	2.78	10	0	2.77±0.14	5
	C		2.49	2.50	10	10	2.50±0.22	10
	A	3.73	3.67	3.68	20	20	3.68±0.12	20
	B		3.76	3.79	10	10	3.78±0.17	10
	C		3.65	3.64	30	30	3.65±0.16	30
	A	4.23	4.19	4.22	50	30	4.21±0.11	40
	B		4.25	4.34	20	40	4.30±0.25	30
	C		4.15	4.21	40	30	4.18±0.20	35
	A	5.23	5.11	5.07	60	70	5.09±0.21	65
	B		5.16	5.19	50	30	5.18±0.10	40
	C		5.12	5.01	70	50	5.07±0.18	60
	A	6.23	6.02	6.02	70	60	6.02±0.18	65
	B		6.00	6.04	80	60	6.02±0.14	70
	C		5.89	5.93	70	80	5.91±0.25	75
	A	6.73	6.47	6.50	90	80	6.49±0.15	85
	B		6.37	6.34	90	80	6.36±0.16	85
	C		6.50	6.38	90	90	6.44±0.30	90

Appendix 3

Physical and Chemical Properties of Water in Sublethal Experiments

Characteristic	Unit	N	$\bar{X}$ ± 1Sd
pH	-	231	7.2± 1.0
Total hardness	as $CaCO_3$, mg/l	40	89.6±19.1
Total alkalinity	as $CaCO_3$, mg/l	40	16.4± 3.9
Dissolved oxygen	mg/l	72	6.6± 0.6
Temperature	°C	229	25.9± 0.7
Total zinc	mg/l		
(Nominal)			
1.93	mg/l	78	1.70±0.15
0.97	mg/l	90	0.88±0.09
0.48	mg/l	77	0.36±0.04

Appendix 4

Size of Females Used in Sublethal Tests

Group	Zinc concentration (measured, mg/l)	Size of female (Standard length, mm)	$\bar{X}$ ± 1Sd
A	0	35.8	
		34.8	
		32.3	32.8±2.5
		30.2	
		30.7	
B	0.36	38.0	
		32.2	
		33.2	32.8±3.3
		31.4	
		29.2	
C	0.88	33.3	
		41.2	
		32.9	33.7±4.4
		30.9	
		30.2	
D	1.70	35.6	
		30.7	
		35.3	34.1±2.8
		37.2	
		31.7	

Appendix 5

Intervals Between Broods (in days) of Female Guppies During Chronic Exposure to Zinc

Zinc concentrations (measured, mg/l)	Females	Parturition Periods			
		1	2	3	4
0	1	33	35	38	28
	2	27	38	44	31
	3	31	33	37	40
	4	36	37	29	35
	5	39	33	30	33
		$\bar{X}$ =33.2	35.2	35.6	33.4
0.36	1	Died			
	2	39	26	28	36
	3	29	32	37	43
	4	28	27	33	29
	5	32	39	33	34
		$\bar{X}$ =32	31	32.8	35.5
0.88	1	28	39	33	40
	2	36	30	40	32
	3	27	33	36	37
	4	30	42	30	33
	5	25	34	35	45
		$\bar{X}$ =29.2	35.6	34.8	37.4
1.70	1	30	35	43	28
	2	39	40	34	31
	3	25	35	36	38
	4	23	33	30	26
	5	30	died		
		$\bar{X}$ =29.4	35.8	35.8	30.8

Appendix 6

Effect of Chronic Exposure to Zinc on Brood Size of the Guppy

Zinc concentrations (measured, mg/l)	Females	Parturition Period				Total	$\bar{X}$
		1	2	3	4		
0	1	14	28	26	18	86	21.5
	2	11	44	12	14	81	20.25
	3	16	11	25	20	72	18.0
	4	35	28	33	36	132	33.0
	5	6	32	4	12	54	13.5
	Total=	82	143	100	100	425	106.25
	$\bar{X}$ =	16.4	28.6	20	20	85	21.25
0.36	1	Died					
	2	5	25	8	10	48	12
	3	16	12	5	6	39	9.8
	4	22	30	28	13	93	23.3
	5	17	22	20	18	77	19.3
	Total=	60	89	61	47	257	64.4
	$\bar{X}$ =	15	22.3	15.3	11.8	64.3	16.1
0.88	1	20	4	3	5	32	8
	2	24	14	5	8	51	12.8
	3	13	2	6	3	24	6
	4	33	2	3	3	41	10.3
	5	18	5	4	2	29	7.3
	Total=	108	27	21	21	177	44.4
	$\bar{X}$ =	21.6	5.4	4.2	4.2	35.4	8.9
1.70	1	26	8	10	6	50	12.5
	2	29	6	7	4	46	11.5
	3	35	3	2	10	50	12.5
	4	9	1	8	5	23	5.8
	5	27	Died				
	Total=	126	18	27	25	169	42.3
	$\bar{X}$ =	25.2	4.5	6.8	6.3	42.3	11.5

Appendix 7

Effect of Chronic Exposure to Zinc on Size (=standard length, mm) of Young at Birth

Zinc concentration = 0 mg/1

Females	1st Parturition	2nd Parturition	3rd Parturition	4th Parturition
1	7.0	6.8	6.5	5.8
	6.6	7.0	5.3	7.3
	6.7	6.4	6.0	7.0
	6.5	5.4	5.8	6.8
	5.9	5.9	6.8	7.6
	7.4	6.3	6.4	8.1
	7.3	6.9	5.0	6.0
	6.8	6.4	7.3	6.5
	6.9	5.8	6.9	7.6
	6.9	7.1	6.0	7.5
2	6.8	7.3	7.8	7.0
	7.3	6.8	7.4	6.9
	7.5	7.0	8.0	6.5
	6.9	6.5	7.6	8.2
	7.6	5.8	6.8	7.6
	7.3	6.6	6.5	7.2
	6.5	6.0	7.3	6.8
	7.0	5.5	5.8	5.0
	6.3	5.4	7.6	6.2
	7.9	6.4	8.0	6.6
3	5.9	7.0	7.0	5.6
	6.8	7.6	7.8	5.4
	5.4	6.5	6.4	5.0
	5.8	5.0	5.6	5.8
	6.5	5.8	6.6	7.1
	6.0	5.5	6.0	5.3
	6.3	6.8	8.1	6.0
	6.0	7.0	7.0	6.8
	6.6	7.3	5.9	6.6
	7.2	6.4	5.5	6.3
4	7.2	7.2	6.8	7.0
	6.0	6.6	7.2	6.3
	6.9	5.8	7.4	6.9
	5.8	6.3	5.8	6.7
	6.8	6.4	7.3	5.8
	7.4	6.5	6.5	7.6
	6.5	5.0	6.0	7.2
	7.3	7.3	7.0	5.6
	6.0	7.0	7.5	6.3
	5.9	6.7	6.0	6.9
5	7.4	7.8	6.5	5.8
	8.0	7.6	6.0	5.4
	6.6	6.5	6.8	6.0
	5.8	6.3	6.3	7.3
	6.0	5.8	6.7	6.6
	6.4	5.4	5.6	6.1
	5.5	5.0	5.8	7.0
	6.6	6.6	6.6	5.6
	6.3	7.3	5.4	6.3
	7.0	7.5	7.0	6.9
	$\bar{X}$ = 6.7	6.5	6.6	6.6

Appendix 7 Cont'd

Zinc concentration = 0.36 mg/l

Females	1st Parturition	2nd Parturition	3rd Parturition	4th Parturition
2	7.0 7.0 6.8 6.9 6.5	5.6 7.2 6.3 6.8 6.0 6.3 5.8 5.7 6.6 6.4	6.2 7.3 6.5 6.4 5.8 7.0 6.3 5.9	6.3 5.2 6.3 6.5 6.0 5.4 7.0 6.6 6.2 5.8
3	6.5 7.0 6.3 5.8 6.4 6.8	8.2 7.1 6.8 6.0 5.4 5.9 6.5 6.8 7.0 5.7	6.8 7.0 6.4 6.5 6.3	6.0 6.8 5.8 6.6 5.8 6.6
4	6.6 5.8 6.3 5.4 7.2 6.4 5.2 5.8 6.1 5.0	6.0 5.3 6.6 5.9 6.0 6.8 6.3 6.0 7.0 5.2	8.1 8.3 7.2 7.6 6.0 6.8 6.9 7.0 6.4 7.5	7.7 8.0 7.1 6.6 5.4 6.3 6.9 7.2 6.0 6.8
5	7.2 6.8 7.0 7.6 6.4 6.9 7.5 7.4 6.7 6.3	6.6 6.7 6.4 7.3 6.6 5.7 6.8 5.6 6.0 6.2	7.6 5.9 6.0 7.3 5.9 6.6 7.4 6.8 7.6 6.5	5.9 6.6 6.0 6.4 6.0 6.3 7.0 6.2
	$\overline{X}$ = 6.5	6.3	6.8	6.4

Appendix 7 Cont'd

Zinc concentration = 0.88 mg/l

Females	1st Parturition	2nd Parturition	3rd Parturition	4th Parturition
1	5.5	5.0	6.0	7.1
	5.8	5.6	5.4	6.4
	6.6	5.4	6.0	6.0
	7.8	5.2		5.5
	5.6			6.5
	6.5			
	6.8			
	7.0			
	6.5			
	6.9			
2	6.3	5.1	6.8	4.5
	6.0	4.3	7.0	4.8
	5.8	4.6	6.0	6.3
	5.4	4.0	5.5	4.6
	6.6		8.2	5.4
	7.0			5.0
	6.2			4.5
	6.3			4.9
	6.7			
	6.3			
3	4.9	5.6	5.7	5.6
	4.6	5.0	6.2	5.9
	5.8		6.0	7.1
	5.5		5.0	
	6.0		4.8	
	5.7		5.9	
	6.3			
	5.9			
	6.1			
	5.7			
4	6.5	6.0	6.4	5.9
	5.4	5.2	5.6	4.5
	6.8		6.0	5.2
	5.0			
	6.1			
	5.0			
	5.5			
	6.3			
	6.0			
	6.3			
5	5.6	5.0	7.1	5.8
	7.0	5.7	5.2	7.0
	6.6	6.3	6.3	
	5.5	5.5	5.0	
	6.0	5.0		
	5.9			
	6.4			
	4.2			
$\bar{X}$ =	6.1	5.2	6.0	5.6

Appendix 7 Cont'd

Zinc concentration = 1.70 mg/l

Females	1st Parturition	2nd Parturition	3rd Parturition	4th Parturition
1	6.0	6.0	6.3	4.4
	6.4	6.8	5.4	5.3
	5.4	6.3	6.0	6.0
	6.2	5.5	5.2	4.5
	6.6	6.4	5.0	5.0
	5.3	7.0	6.6	4.8
	5.0	6.6	5.8	
	7.0	6.0	6.0	
	5.4		5.5	
	6.0		6.0	
2	7.0	5.3	7.0	5.8
	6.4	4.4	5.0	6.0
	7.3	4.1	5.5	6.3
	6.9	5.6	6.0	5.9
	6.3	5.0	4.8	
	6.0	5.6	5.8	
	6.8		6.6	
	6.8			
	6.6			
	7.0			
3	6.5	6.0	6.8	4.6
	6.7	5.6	6.0	4.9
	6.1	6.4		5.3
	5.3			6.0
	6.0			6.2
	5.5			5.5
	6.0			6.3
	5.2			5.8
	5.6			6.0
	6.8			5.6
4	4.9	4.8	4.8	3.7
	5.6		4.6	4.0
	6.0		5.3	4.3
	5.8		4.4	4.0
	6.6		6.0	4.5
	5.5		5.0	
	6.5		4.2	
	6.7		6.0	
	4.8			
5	5.0	Died		
	6.5			
	4.9			
	7.3			
	6.6			
	6.0			
	5.5			
	5.8			
	6.0			
	6.7			
	$\bar{X}$ = 6.1	5.7	5.6	5.2

Appendix 8

Mean Weekly Courtship Behaviour Scores of Male Guppies (per 3 minutes) During Chronic Exposure to Zinc

S = Sigmoid reaction
D = dorsal fin erection
G = gonopodial swinging
T = total score

Zinc concentration (measured, mg/l)	Males	Weeks											
		1				2				3			
		S	D	G	T	S	D	G	T	S	D	G	T
0	1	9.3	7.7	1.0	18.0	9.3	8.0	1.0	18.3	6.3	6.3	1.3	13.9
	2	4.7	5.7	1.7	12.1	5.7	2.7	1.7	10.1	5.0	7.3	1.0	13.3
	3	8.0	6.0	1.0	15.0	17.0	13.3	2.0	32.3	10.7	2.0	1.0	13.7
	4	7.0	6.3	0.3	13.6	18.0	10.3	2.0	30.3	9.0	2.0	2.0	13.0
	5	6.3	5.0	0.3	11.6	12.3	13.0	1.3	26.6	2.0	3.7	0.3	6.0
		Overall $\overline{X}$ =								8.7	6.6	1.2	16.5
0.36	1	7.3	6.3	3.7	17.3	5.3	6.3	1.3	12.9	3.7	6.3	0.0	10.0
	2	6.0	5.0	1.0	12.0	10.7	4.7	1.7	17.1	8.0	5.7	1.3	15.0
	3	9.0	8.7	1.0	18.7	17.7	15.7	1.0	34.4	9.7	3.0	1.7	14.4
	4	8.0	7.0	2.0	17.0	6.0	9.7	1.3	17.0	10.3	5.3	0.3	15.9
	5	3.3	7.7	0.7	11.7	3.0	5.7	2.7	11.4	3.0	4.3	0.7	8.0
		Overall $\overline{X}$ =								7.4	6.8	1.4	15.5
0.88	1	10.3	8.7	1.0	20.0	5.7	5.0	0.0	10.7	6.3	7.0	0.3	13.6
	2	7.7	6.0	1.0	14.7	8.7	8.3	3.7	20.7	6.7	5.0	1.0	12.7
	3	9.0	8.3	2.0	19.3	10.3	5.3	1.7	17.3	7.0	7.0	0.3	14.3
	4	8.3	6.3	2.0	16.6	17.0	15.7	1.0	33.7	11.0	2.3	1.0	14.3
	5	4.3	3.3	0.7	8.3	4.0	5.7	2.0	11.7	4.3	5.3	0.7	10.3
		Overall $\overline{X}$ =								8.0	6.6	1.2	15.9
1.70	1	6.0	6.3	1.7	14.0	4.7	5.3	1.0	11.0	3.3	4.0	1.3	8.6
	2	11.3	8.0	1.0	20.3	8.0	8.7	2.7	19.4	7.3	7.0	0.7	15.0
	3	8.3	9.0	0.7	18.0	8.7	8.7	2.3	19.7	6.7	6.0	1.0	13.7
	4	7.3	8.0	1.3	16.6	8.3	8.0	1.7	18.0	9.0	4.0	0.7	13.7
	5	4.3	8.7	1.3	14.3	5.0	6.0	0.7	11.7	5.7	2.0	0.7	8.4
		Overall $\overline{X}$ =								6.9	6.6	1.3	14.8

Appendix 8 Cont'd

Zinc concentration (measured, mg/l)	Males	Weeks											
		4				5				6			
		S	D	G	T	S	D	G	T	S	D	G	T
0	1	4.7	6.7	2.3	13.7	6.0	6.0	2.3	14.3	5.3	7.7	2.0	15.0
	2	4.7	5.0	2.0	11.7	4.3	5.0	1.0	10.3	8.0	4.7	1.7	14.4
	3	8.3	3.7	2.0	14.0	4.0	3.3	2.0	9.3	8.0	2.3	1.7	12.0
	4	5.0	4.7	0.7	10.4	3.3	5.7	0.3	9.3	3.0	4.7	1.3	9.0
	5	4.7	2.7	1.3	8.7	8.3	5.0	0.7	14.0	3.0	3.3	1.0	7.3
		Overall $\overline{X}$ =								5.4	4.7	1.5	11.6
0.36	1	3.0	6.3	1.7	11.0	6.7	3.3	1.0	11.0	Discontinued			
	2	9.3	5.3	2.7	17.3	3.3	7.0	1.0	11.3	5.3	5.0	1.7	12.0
	3	4.0	4.7	1.3	10.0	5.0	4.7	1.7	11.4	8.0	5.3	2.0	15.3
	4	6.0	2.7	1.0	9.7	8.0	4.7	1.0	13.7	6.3	3.0	0.7	10.0
	5	3.7	4.0	1.3	9.0	1.7	4.7	1.3	7.7	3.3	2.7	0.3	6.3
		Overall $\overline{X}$ =								5.3	4.5	1.3	11.1
0.88	1	7.0	6.0	1.7	14.7	7.0	5.0	2.0	14.0	8.3	5.0	2.3	15.6
	2	5.7	4.7	1.3	11.7	8.3	5.7	1.7	15.7	8.3	5.3	1.3	14.9
	3	6.7	3.3	2.3	12.3	6.0	5.0	1.3	12.3	5.3	5.7	2.3	13.3
	4	3.7	4.7	2.0	10.4	1.7	4.0	0.3	6.0	4.3	5.3	0.7	10.3
	5	3.0	2.7	0.3	6.0	4.0	3.0	0.3	7.3	3.3	3.3	0.7	7.3
		Overall $\overline{X}$ =								5.5	4.6	1.4	11.5
1.70	1	6.7	6.7	1.7	15.1	5.3	4.7	1.7	11.7	6.0	4.0	1.7	11.7
	2	5.7	7.0	2.0	14.7	7.0	6.0	1.7	14.7	6.7	4.7	1.3	12.7
	3	6.3	2.3	1.0	9.6	5.7	3.0	1.0	9.7	4.3	3.3	1.3	8.9
	4	4.7	1.7	0.7	7.1	3.7	3.3	0.7	7.7	2.7	4.3	2.0	9.0
	5	2.0	3.0	1.7	6.7	3.0	3.3	1.0	7.3	4.3	4.3	1.3	9.9
		Overall $\overline{X}$ =								4.9	4.1	1.4	10.4

Appendix 8 Cont'd

Zinc concentration (measured, mg/l)	Males	Weeks											
		7				8				9			
		S	D	G	T	S	D	G	T	S	D	G	T
0	1	6.3	1.0	2.3	9.6	5.3	4.3	2.0	11.6	7.0	7.7	1.0	15.7
	2	5.7	4.7	2.7	13.1	9.0	5.3	1.3	15.6	5.7	2.3	0.7	8.7
	3	9.7	3.0	1.7	14.4	6.7	3.3	2.3	12.3	6.3	6.0	3.7	16.0
	4	2.0	5.0	1.3	8.3	2.7	4.7	1.0	8.4	8.3	5.3	1.3	14.9
	5	6.0	3.7	1.7	11.4	2.3	3.3	0.7	6.3	1.3	5.3	0.7	7.3
		Overall $\overline{X}$ =								5.6	4.3	1.6	11.6
0.36	2	4.0	4.7	1.7	10.4	6.3	5.3	1.7	13.3	7.3	8.0	2.0	17.3
	3	8.0	5.3	1.0	14.3	4.3	3.0	1.0	8.3	7.0	1.3	2.0	10.3
	4	4.7	4.3	1.0	10.0	1.0	4.0	1.3	6.3	4.7	6.3	2.0	13.0
	5	8.7	3.3	1.0	13.0	5.3	4.7	2.0	12.0	4.7	2.3	1.0	8.0
		Overall $\overline{X}$ =								5.5	4.4	1.5	11.4
0.88	1	5.0	3.0	0.7	8.7	5.3	6.0	0.7	12.0	8.0	5.7	1.3	15.0
	2	7.3	2.3	0.3	9.9	6.0	6.0	1.7	13.7	6.3	5.3	1.7	13.3
	3	5.3	5.0	1.3	11.6	5.0	6.7	2.3	14.0	5.7	3.7	1.0	10.4
	4	7.7	3.3	2.0	13.0	3.7	3.7	0.3	7.7	4.0	6.3	2.0	12.3
	5	3.3	4.0	0.0	7.3	1.7	4.0	0.7	6.4	5.0	5.7	1.7	12.4
		Overall $\overline{X}$ =								5.3	4.7	1.2	11.2
1.70	1	4.7	4.0	2.0	10.7	7.0	7.0	2.0	16.0	5.0	5.0	2.7	12.7
	2	4.0	4.0	0.3	8.3	5.0	4.3	0.7	10.0	5.3	4.3	0.0	9.6
	3	8.0	5.7	0.3	14.0	5.3	1.3	1.0	7.6	5.3	3.3	2.3	10.9
	4	6.7	2.0	1.0	9.7	4.0	3.7	1.7	9.4	5.0	4.3	0.7	10.0
		Overall $\overline{X}$ =								5.4	4.1	1.2	10.7

Appendix 8 Cont'd

Zinc concentration (measured, mg/l)	Males	Weeks											
		10				11				12			
		S	D	G	T	S	D	G	T	S	D	G	T
0	1	7.0	5.0	0.7	12.7	5.0	7.7	2.3	15.0	5.7	3.7	2.3	11.7
	2	2.0	6.3	3.3	11.6	6.0	6.7	2.0	14.7	7.7	6.0	1.3	15.0
	3	8.3	7.7	1.3	17.3	4.3	4.0	1.7	10.0	5.3	3.3	0.7	9.3
	4	4.3	5.0	0.7	10.0	7.0	2.7	1.0	10.7	7.3	5.0	2.7	15.0
	5	2.7	4.0	2.0	8.7	4.0	2.3	1.7	8.0	4.0	0.7	1.3	6.0
		Overall $\bar{X}$ =								5.4	4.7	1.7	11.7
0.36	2	6.7	3.3	1.7	11.7	7.0	6.7	1.3	15.0	7.7	5.3	1.7	14.7
	3	4.3	9.3	0.7	14.3	6.0	3.7	1.0	10.7	6.0	3.7	2.0	11.7
	4	2.7	3.3	2.7	8.7	3.3	6.0	2.0	11.3	5.3	2.7	1.3	9.3
	5	4.7	6.3	1.3	12.3	6.0	4.7	1.7	12.4	3.3	2.3	1.0	6.6
		Overall $\bar{X}$ =								5.3	4.8	1.5	11.6
0.88	1	7.0	6.0	1.0	14.0	5.0	6.3	2.7	14.0	5.7	6.3	2.3	14.3
	2	4.3	7.0	3.3	14.6	9.0	4.3	0.3	13.6	6.7	6.0	0.3	13.0
	3	6.3	5.3	0.7	12.3	1.7	5.7	1.7	9.1	6.3	4.7	1.7	12.7
	4	8.0	2.7	1.3	12.0	7.7	6.3	0.7	14.7	5.7	3.0	1.7	10.4
	5	2.0	0.3	1.3	3.6	3.3	4.0	1.7	9.0	4.0	1.3	1.3	6.6
		Overall $\bar{X}$ =								5.5	4.6	1.5	11.6
1.70	1	6.7	4.3	2.0	13.0	7.0	4.0	1.3	12.3	6.7	4.0	2.0	12.7
	2	5.0	4.7	1.7	11.4	4.7	7.0	1.0	12.7	6.3	5.0	1.7	13.0
	3	8.0	6.3	2.0	16.3	7.3	5.7	1.0	14.0	6.0	5.0	1.3	12.3
	4	4.3	7.0	1.7	13.0	6.3	3.0	0.7	10.0	4.3	2.0	1.0	7.3
		Overall $\bar{X}$ =								6.1	4.8	1.5	12.3

Appendix 9

Number of Times Horizontal Lines were Crossed x 0.7
(= measure of movement in the vertical plane)

Zinc concentration (measured, mg/l)	Females	Weeks					
		1	2	3	4	5	6
0	1	14.0	14.5	16.6	12.4	4.4	6.3
	2	13.5	6.1	7.2	5.1	2.1	4.9
	3	7.0	11.7	10.5	11.9	12.4	14.2
	4	6.1	9.1	7.9	7.9	3.7	17.3
	5	9.3	10.3	6.3	8.4	2.1	12.6
	$\bar{x}$			10.0			8.4
0.36	1	5.4	6.5	5.6	2.1	4.4	Died
	2	6.1	3.7	4.7	6.1	5.8	2.8
	3	7.2	7.7	7.2	3.0	1.9	2.3
	4	4.0	5.8	5.4	6.3	0.9	2.6
	5	8.4	7.0	6.5	2.1	0.5	4.2
	$\bar{x}$			6.1			3.2
0.88	1	14.0	7.7	5.1	4.9	1.9	1.4
	2	3.5	6.8	2.6	2.1	2.6	4.0
	3	9.3	5.1	4.9	4.2	1.6	1.6
	4	4.9	2.3	5.4	1.9	3.5	4.7
	5	6.1	7.0	7.5	6.1	2.1	3.3
	$\bar{x}$			6.1			3.1
1.70	1	9.1	11.7	2.6	0.0	0.2	1.4
	2	7.0	5.6	0.9	1.9	0.7	1.2
	3	6.8	8.6	2.1	0.0	0.2	2.1
	4	7.9	10.3	7.0	0.2	1.2	0.5
	5	4.7	7.2	4.7	3.5	1.9	3.0
	$\bar{x}$			6.4			1.2

Appendix 9 Cont'd

Zinc concentration (measured, mg/l)	Females	Weeks 7	8	9	10	11	12
0	1	11.0	4.7	4.9	5.6	6.3	5.6
	2	3.5	5.1	4.0	5.1	4.0	5.6
	3	9.8	5.6	4.4	3.0	4.2	9.6
	4	11.0	7.2	7.0	9.6	4.2	5.6
	5	11.0	4.0	7.7	4.7	5.1	9.8
	$\overline{X}$			6.7			5.9
0.36	2	3.5	1.9	5.4	4.7	3.3	3.7
	3	12.8	6.3	4.4	3.5	5.1	2.3
	4	5.6	7.7	5.1	4.9	4.0	4.2
	5	1.4	3.5	5.1	3.3	7.9	6.0
	$\overline{X}$			5.2			4.4
0.88	1	1.9	2.8	4.0	1.2	1.6	2.6
	2	0.7	0.9	1.6	0.9	1.6	2.1
	3	2.1	2.1	0.7	0.7	0.9	0.7
	4	3.7	2.6	3.3	0.9	5.6	3.5
	5	6.1	2.6	2.6	3.5	1.4	3.0
	$\overline{X}$			2.5			2.0
1.70	1	0.9	0.7	2.6	1.4	4.0	4.2
	2	3.0	1.2	1.4	1.4	0.2	1.4
	3	0.5	0.9	0.7	0.7	1.4	2.8
	4	2.8	1.2	1.9	1.2	1.6	2.3
	5	Died					
	$\overline{X}$			1.5			1.9

Appendix 10

Number of Times Vertical Lines were Crossed
(= measure of movement in the horizontal plane)

Zinc concentration (measured, mg/l)	Females	Weeks					
		1	2	3	4	5	6
0	1	30.3	38.3	35.0	21.7	35.3	33.3
	2	34.7	40.7	40.3	41.7	38.0	37.3
	3	25.7	34.0	39.0	59.0	55.7	52.0
	4	35.3	33.7	50.3	38.3	41.7	50.0
	5	22.7	30.0	40.7	30.7	24.0	36.7
	$\bar{X}$			35.4			39.7
0.36	1	36.7	30.3	41.0	17.7	19.7	Died
	2	28.3	30.3	34.3	29.0	31.3	25.3
	3	39.0	40.6	30.7	26.0	16.3	12.7
	4	26.0	28.3	38.3	21.0	8.7	17.0
	5	32.3	37.0	33.5	17.7	20.0	10.0
	$\bar{X}$			33.8			19.5
0.88	1	34.3	37.3	30.3	12.0	9.7	20.7
	2	25.7	28.3	28.0	24.7	23.7	8.7
	3	28.0	36.3	18.7	21.7	4.0	10.0
	4	33.3	36.0	30.3	12.0	27.0	14.3
	5	35.0	46.7	36.7	31.0	19.3	26.3
	$\bar{X}$			32.3			17.7
1.70	1	36.0	38.0	30.3	8.0	4.3	6.0
	2	26.7	25.3	28.0	12.0	8.0	10.0
	3	31.3	33.7	20.3	11.3	10.0	6.7
	4	33.3	33.0	24.7	2.3	2.7	7.7
	5	35.7	26.0	18.0	16.0	5.7	13.7
	$\bar{X}$			29.4			8.3

Appendix 10 Cont'd

Zinc concentration (measured, mg/l)	Females	Weeks 7	8	9	10	11	12
0	1	28.0	20.3	29.3	27.7	18.0	20.0
	2	45.7	20.3	39.3	34.7	30.3	32.5
	3	53.0	55.7	51.3	44.0	54.7	40.7
	4	45.0	52.3	48.0	47.0	39.0	35.0
	5	33.3	36.0	39.0	33.7	56.3	40.6
	$\overline{X}$			39.8			36.9
0.36	2	18.3	8.7	24.3	17.7	26.0	20.0
	3	31.3	28.3	27.7	21.0	27.6	24.6
	4	18.0	23.7	23.7	35.0	18.3	22.3
	5	9.7	15.3	34.7	26.3	34.7	20.3
	$\overline{X}$			22.0			24.5
0.88	1	21.3	21.3	25.0	19.3	12.0	16.0
	2	3.3	5.7	20.3	22.3	20.7	20.0
	3	14.7	11.3	13.3	7.0	8.3	10.0
	4	16.3	9.7	24.7	7.0	25.7	12.3
	5	24.0	16.7	25.3	30.7	19.3	20.0
	$\overline{X}$			16.9			16.7
1.70	1	14.7	12.7	11.0	16.0	16.3	14.7
	2	13.0	9.3	13.0	4.7	9.0	10.0
	3	12.3	9.7	7.0	11.0	7.7	8.0
	4	19.3	7.0	9.0	5.0	4.3	5.0
	5	Died					
	$\overline{X}$			11.5			9.3

Appendix 11

Number of Times Horizontal Lines were Crossed and Number of Times Vertical Lines were Crossed (= measure of total movement)

Zinc concentration (measured, mg/l)	Females	Weeks					
		1	2	3	4	5	6
0	1	44.3	52.8	51.6	34.1	39.7	39.6
	2	48.2	46.7	47.5	46.8	40.1	42.2
	3	32.7	45.7	49.5	70.9	68.1	66.2
	4	41.4	42.8	58.2	46.2	45.4	67.3
	5	32.0	40.3	47.0	39.0	26.1	49.3
	$\bar{X}$			45.4			48.1
0.36	1	42.1	36.8	46.6	19.8	24.1	Died
	2	34.4	34.0	39.0	35.1	37.1	28.1
	3	46.2	48.3	37.9	29.0	18.2	15.0
	4	30.0	34.1	43.7	27.3	9.6	19.6
	5	40.7	44.0	40.0	19.8	20.5	14.2
	$\bar{X}$			39.9			22.7
0.88	1	48.3	45.0	35.5	16.9	11.6	22.1
	2	29.2	35.1	30.5	26.8	26.3	12.7
	3	37.3	41.4	23.7	25.9	5.6	11.6
	4	38.2	38.3	35.7	13.9	30.5	19.0
	5	41.1	53.7	44.2	37.1	21.4	29.6
	$\bar{X}$			38.5			20.7
1.70	1	45.1	49.7	32.9	8	4.5	7.4
	2	33.7	30.9	28.9	13.9	8.7	11.2
	3	38.1	42.3	22.4	11.3	10.2	8.8
	4	41.2	43.3	31.7	2.5	3.9	8.2
	5	40.4	33.2	22.7	19.5	7.6	16.7
	$\bar{X}$			35.8			9.5

Appendix 11 Cont'd

Zinc concentration (measured, mg/l)	Females	Weeks					
		7	8	9	10	11	12
0	1	38.7	25.0	34.2	33.3	26.3	25.6
	2	49.2	25.4	43.3	39.8	34.3	38.1
	3	62.8	61.3	55.7	47.0	58.9	50.3
	4	53.0	59.5	55.0	56.6	43.2	40.6
	5	44.2	40	46.7	38.4	61.4	50.4
	$\bar{X}$			46.3			42.9
0.36	2	21.8	10.6	29.7	22.4	29.3	23.7
	3	44.1	34.6	32.1	25.4	32.7	26.9
	4	23.6	31.4	28.8	39.9	22.3	26.5
	5	11.1	18.8	39.8	29.6	42.6	26.0
	$\bar{X}$			27.2			28.9
0.88	1	23.2	24.1	29.0	20.5	13.6	18.6
	2	4.0	6.6	21.9	23.2	22.3	22.1
	3	16.8	13.4	14.0	7.7	9.2	10.7
	4	20.0	12.3	28.0	7.9	31.3	15.8
	5	30.1	19.3	27.9	34.2	20.7	23.0
	$\bar{X}$			19.4			18.7
1.70	1	15.6	13.4	13.6	17.4	20.3	18.9
	2	16.0	10.5	14.4	6.1	9.2	11.4
	3	12.8	10.6	7.7	11.7	9.1	10.8
	4	22.1	8.2	10.9	6.2	5.9	7.4
	5	Died					
	$\bar{X}$			13.0			11.2

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